



Development and application of systematic clinical udder examinations as supplementary tool in udder health assessment

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**Development and Application of
Systematic Clinical Udder Examinations as
Supplementary Tool in Udder Health Assessment**

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Preface

This thesis is intended to fulfil the requirements for the Ph.D. degree at the Royal Veterinary and Agricultural University, Copenhagen, Denmark. The research was carried out from 2003-2005 at the Danish Institute of Agricultural Sciences (DIAS), Department of Animal Health, Welfare and Nutrition. A part of the research is based on data I collected in Germany during 1997 and 1998. Prof. Edgar Schallenger from the Christian-Albrechts-University in Kiel, Germany, is acknowledged for the kind permission to analyse the data.

It was a challenge and a great experience to examine cows in different study populations in Germany and in Denmark. As implied by the drawing on the front page - cows come in all shapes and sizes. After having examined so many of them, I'm still amazed over the variety of conditions, making it a real challenge to use the information gathered with eyes and hands in a systematic way.

A number of people have inspired and supported me during the project. First of all, I would like to thank my supervisor Carsten Enevoldsen and co-supervisor Mette Vaarst for continuous support in all stages of the project, inspiring discussions, and patience. Carsten taught me that statistics is not as threatening as it may appear, and he always had a solution at hand when I was stuck with the numbers. Mette with her holistic approach always put me on track again when I couldn't see where I was going. Both always focussed on the real life relevance of the research.

I am very grateful to my co-authors for their valuable inputs and suggestions. I would also like to thank my colleagues from the Research Unit of Herd Health and Production Management at DIAS for their support and friendship. Enrollment in the Research School for Animal Production and Health (RAPH) gave me the opportunity for intensive discussions of all aspects of science. It was a great experience to carry out research in commercial dairy farms. I am very grateful to the farmers for their cooperation, hospitality and patience, the latter particularly relevant when I examined thousands of udders at five o'clock something in the morning in their milking parlours. The fruitful discussions gave me as a veterinarian insight in the farmers' side of the mastitis story and inspired my work.

Finally, a big thank you to my family and friends for their support and understanding.

Foulum, November 2005

Ilka Christine Klaas

Contents

Summary	9
Sammendrag.....	13
1 Introduction	18
1.1 Background.....	18
1.2 Objective of the thesis	19
1.2.1 Specific objectives of the subprojects.....	20
1.3 Outline of the thesis.....	20
2. Literature	21
2.1 Pathogenesis of mastitis	21
2.2 Definition of mastitis and udder health.....	22
2.2.1 Treated cases of mastitis	22
2.2.2 Clinical mastitis	23
2.2.3 Subclinical mastitis	23
2.2.4 Healthy udder.....	24
2.3 Methods to assess udder health status based on monitoring constituents of the milk ...	25
2.3.1 Somatic cell count on quarter level.....	25
2.3.2 Cow somatic cell counts from milk-recording.....	25
2.3.3 Bulk milk somatic cell count (BMSCC).....	26
2.3.4 Identification of mastitis pathogens	27
2.3.5 Other indicators.....	27
2.4 Systematic clinical examinations in udder health assessment	28
2.4.1 Definition of Systematic Clinical Examinations.....	28
2.4.2 Clinical udder examination	30
2.4.3 Morphologic features (traits).....	31
2.4.4 Milking related and environmental changes	32
2.4.5 Pathologic changes caused by infections	32
2.4.6 How to do systematic clinical examinations support udder health monitoring ...	33

3 Materials and Methods	35
3.1 Study populations and study design	35
3.2 Data sources	37
3.2.1 Monthly milk recording	37
3.2.2 Mastitis treatments	37
3.3 Systematic clinical udder examinations	37
3.4 Statistical analysis	39
3.4.1 Logistic regression with random effects	39
3.4.2 Principal component analysis	41
3.4.3 Modelling of the lactation curve	43
4 Results	45
4.1 Cow-level risk factors for milk leakage	45
4.2 Risk Factors for Intramammary Infections (IMI) with <i>Strep. uberis</i> and <i>Staph. aureus</i> and Clinical Mastitis (CM) in First Lactation Cows	45
4.2.1 IMI with <i>Streph. uberis</i>	45
4.2.2. IMI with <i>Streph. aureus</i>	46
4.2.3 Clinical mastitis	46
4.3 Systematic Clinical Examinations for Identification of Latent Udder Health Types	46
4.4 Udder health parameters in organic farms with non-antibiotic treatment strategy	48
5 Discussion	51
5.1 Study design and data collection	51
5.1.1 The clinical variables	51
5.1.2 Observational studies	51
5.1.3 Study design and adjustments according to practical circumstances	53
5.1.4 Clinical variables and observer effect	53
5.1.5 Automatic milking systems as example for adjustment of SCE according to practical circumstances	54

5.2 Challenges in statistical modelling	55
5.2.1 Quarter level	55
5.2.2 Definition of cases of clinical mastitis.....	55
5.3 Udder health indicators for udder health assessment	58
5.3.1 Chronic udder type	58
5.3.2 Principal component analysis to identify patterns	58
5.3.3 Hygiene score	59
5.3.4 Teat characteristics	59
5.4 Implementation of systematic clinical examinations in udder health monitoring.....	60
6 Conclusion and Future Research	63
6.1 Conclusions	63
6.2 Future research	64
7 References	67
8 Manuscript 1. Cow-related risk factors for milk leakage (Reprint from <i>Journal of Dairy Science</i> 88, 128-136).....	77
9 Manuscript 2. Cow-related risk factors for intramammary infections with <i>Streptococcus uberis</i> and <i>Staphylococcus aureus</i> in primiparous cows (Submitted to <i>Journal of Dairy Science</i>)	87
10 Manuscript 3. Systematic clinical examinations for identification of latent udder types in Danish dairy herds (Reprint from <i>Journal of Dairy Science</i> 87, 1217-1228).....	109
11 Manuscript 4. Udder health parameters and veterinary treatments in Danish organic herds with a non-antibiotic treatment strategy (Submitted to <i>Veterinary Record</i>).....	121

Abbreviation key

AMS	Automatic milking system
AMU	Automatic milking unit
BMSCC	bulk milk somatic cell count
CBSCC	calculated bulk milk somatic cell count
CM	Clinical mastitis
IDF	International Dairy Federation
IMI	Intramammary infection
IMP	Intramammary pressure
SCC	Somatic cell count
SCE	Systematic clinical examination
<i>Staph. aureus</i>	Staphylococcus aureus
<i>Strep. uberis</i>	Streptococcus uberis

Summary

The aim of this thesis was to identify information from systematic clinical udder examinations (SCE) that can be implemented as an additional tool in udder health assessment in dairy farms. Mastitis is the most frequent and costly disease in dairy herds and tremendous efforts are targeted to effective mastitis control. Currently the definition of mastitis varies between studies of herds, regions or countries, which makes it difficult to assess the progress or development in mastitis control. Especially the true incidence of clinical mastitis is difficult to estimate, as we most likely only have access to the antibiotic treated cases of mastitis that are recorded on farms or, as in the Scandinavian countries, in national databases. Mastitis treatments and bulk milk somatic cell counts are highly dependent on the farmer's attitude and thresholds for intervention, and cow somatic cell counts from milk recording alone will give an underestimation of clinical cases of mastitis. With systematic clinical examinations of udders and teats udder health can be assessed independently of farm records. An unambiguously defined sample of cows is clinically examined following a standardized clinical protocol to document the health situation within or across herds, to identify udder health problems and the related risk factors, and to monitor the development of udder health. To explore the potentials of SCE in udder health assessment, this thesis was carried out in different subprojects with the specific objectives to:

- Estimate potentially important relations between major udder disorders (milk leakage, intramammary infections and clinical mastitis) and clinical characteristics of udder and teats.
- Reduce the large number of potentially important udder and teat characteristics related to udder health and production into fewer udder types (identify latent udder health variables).
- Characterize udder health in herds with a specified non-antibiotic treatment policy and use these characteristics to evaluate potentially negative effects of reduced use of antibiotics on udder health.
- Give recommendations for the use of systematic clinical examinations in udder health management with regard to sampling strategy, relevant udder and teat characteristics and statistical analysis.

To achieve these aims, systematic clinical examinations were carried out in three different study populations in Germany and in Denmark. Four manuscripts are included in the thesis:

Manuscript 1 “Cow-related risk factors for milk leakage”

A longitudinal observational study was conducted in 15 German dairy herds. At monthly farm visits milk flow curves were measured during the evening milking. During the subsequent milking, milk leakage was assessed when the cows entered the milking parlour. Teat shape, teat end shape, condition of the teat orifice and udder shape of cows in early lactation (9-100 days in milk) and late lactation (> 250 days in milk) were assessed immediately after detachment of the milking unit. Risk factors for milk leakage were analyzed with a logistic regression model with herd as a random effect. Primiparous cows with high peak milk flow and teat canal protrusions were at higher risk of milk leakage. High peak milk flow rate, short teats, teat canal protrusion, inverted teat ends, and early lactation increased the risk of milk leakage in multiparous cows. Herd accounted for approximately 10 % of the variation in the data indicating that the impact of management or other herd level factors on the occurrence of milk leakage is low.

Manuscript 2 “Cow-related risk factors for intramammary infections with *Streptococcus uberis* and *Staphylococcus aureus* in primiparous cows”

An observational study was conducted in 15 German dairy herds to identify udder and teat characteristics that were related to a higher risk of intramammary infections (IMI) with *Staph. aureus* and *Strep. uberis* and clinical mastitis during the first 100 days of lactation in primiparous dairy cows. Milk samples were collected aseptically at monthly farm visits. The udders and teats of all primiparous cows in early lactation were assessed immediately after milking.

Logistic regression with herd as a random effect showed that deviance from the average milk flow, calving in winter and condition of the teat orifice increased the risk of *Strep.-uberis* infection. The risk of IMI with *Staph. aureus* was higher in cows with short and thick teats, and in cows with pointed teats. Significant risk factors for clinical mastitis were calving in winter, hindquarters, deep udder and large teats. The variance explained by the herd effect in the final models was 2% in the *Strep. uberis*-model, 6% in the *Staph. aureus*-model and 16.7% in the clinical mastitis model.

This study indicated that different udder and teat characteristics are risk factors for IMI with *Strep. uberis* and *Staph. aureus* and for cases of clinical mastitis.

Manuscript 3 “Systematic clinical examinations for identification of latent udder types in Danish dairy herds”

A cross-sectional study was conducted in 16 Danish dairy farms to explore the applicability of systematic clinical examinations of udders as an additional tool in udder health assessment. The farms were visited 5 times during the year 2000 and 20 cows per farm were chosen at random at each visit for clinical udder examination within 2 hours after milking. The clinical examination included morphological and pathological variables. Principal component analysis was used to extract four udder types explaining 30% of the variation of the data: 1) the small udder, 2) the distressed udder, 3) the mastitis udder, and 4) the soiled udder. Variables with high positive correlation to the “small udder” were small udder shape, short teats, and first parity. Impaired teat surface, hard udder texture and a long udder shape were related to the “distressed udder”. The “mastitis udder” was characterized by asymmetry between front or hindquarters, knotty tissue and acute clinical mastitis. Reduced milk yield and high SCC was related to the “mastitis udder” whereas low SCC was related to the “small udder”. The “soiled udder” was related to early lactation. Including this information in the assessment of udder health may be of substantial value for data analysis in farms with suspected under-reporting of clinical mastitis.

Manuscript 4 “Udder health parameters and veterinary treatments in Danish organic herds with a non-antibiotic treatment strategy”

23 organic dairy herds participated in a project with the aim to minimize antibiotic treatments. The udders of 50 cows per farm were clinically examined during milking at the beginning of the project period. The hygiene of body and udder was scored. The data was analysed on herd level using principal component analysis and a general linear model. Principal component analysis extracted two components that may represent two different herd characteristics. Component 1, labelled ‘Dirty, Danish Holsteins & treatments’, was characterized by udder and body hygiene scores, antibiotic mastitis treatments per cow year, asymmetric quarters and Danish Holsteins, whereas component 2 was characterized by nodes in the udder, knotty tissue and blind quarters and labelled ‘chronic udder changes’. Herd leg hygiene score (within component 1) and prevalence of cows with nodes (within component 2) were associated with calculated bulk SCC (CBSCC), explained alone

as much variation as the components and are therefore important udder health indicators. Mastitis treatments per cow year, prevalence of dry quarters and CBSCC were not related, which indicated that a low usage of antibiotics did not have a negative effect on udder health. Herds with Jersey had the lowest usage of antibiotics for mastitis and moderate or low CBSCC (< 300,000). The Jersey herds also had the cleanest cows, but it was not possible to judge whether these results were due to management or breed effects.

General discussion

The subprojects examined morphological and pathological udder and teat characteristics in first parity and older cows, as individual variables and as components. Several udder and teat characteristics were risk factors for milk leakage, pathogen specific intramammary infections and clinical mastitis. Therefore, it will be beneficial to include this information in breeding programs (long-term effect) as well as in farm specific mastitis control plans (short-term effect). Principal component analysis was an effective method to identify patterns within the udder and teat characteristics and in combination with other production data on cow and herd level. The chronic udder type was extracted on herd and cow level, consisted of pathologic changes in the udder and was related to somatic cell count. Under a non-antibiotic treatment policy, antibiotic mastitis treatments, the prevalence of dry quarters and CBSCC were not related, which indicated that a low usage of antibiotics did not have a negative effect on udder health. The method of drying off infected quarters was used as a management tool comparable to using antibiotic treatments. Hence, the prevalence of dry quarters has to be interpreted in relation to other udder health indicators to assess udder health in a specific farm.

In conclusion, systematic clinical examinations of udders contribute to the understanding of udder health, reveal farm specific mastitis treatment patterns and farm specific risk factors. SCE can be used for benchmarking when a consistent and well-defined sampling strategy, and a standardized clinical protocol is used. The implementation of systematic clinical udder examinations will be of special interest in large farms, in farms with suspected under-reporting of mastitis treatments and in farms that want to reduce mastitis treatments.

Sammendrag

Målet med denne Ph.D.afhandling var at identificere information fra systematiske kliniske undersøgelser af yvere (SKU), som kan supplere anden tilgængelig information fra en malkekvægbesætning ved en samlet vurdering af yversundheden.

Mastitis er den hyppigste og mest omkostningsbelastende sygdom i malkekvægbesætninger, og der gøres en meget stor indsats for at kontrollere denne sygdom effektivt. På nuværende tidspunkt varierer definitionen af mastitis mellem besætninger, geografiske områder og lande. Dette vanskeliggør en vurdering af hvorledes situationen udvikler sig, og om der sker forbedringer med hensyn til kontrollen af mastitis. Især er den virkelige forekomst af klinisk mastitis vanskelig at vurdere, fordi vi næsten udelukkende har adgang til information om antibiotikabehandlede mastitistilfælde som enten er registreret på den enkelte gård, eller som i Skandinavien, i nationale databaser. Mastitisbehandlinger og celletallet i den leverede mælk er størrelser, som i høj grad er afhængige af landmandens holdning og kriterier for at gribe ind, mens celletallet hos den enkelte ko ved ydelseskontrollen brugt alene vil føre til en undervurdering af de kliniske mastitistilfælde. Gennemførelsen af systematiske kliniske yverundersøgelser kan vurderes uafhængigt af andre registreringer i besætningen. Undersøgelse af et veldefineret udsnit af en given besætnings kør, hvor man følger en standardiseret klinisk protokol, kan dokumentere sundhedstilstanden i en besætning såvel som mellem forskellige besætninger og dermed bidrage til at identificere problemer med yversundheden og relaterede risikofaktorer, og vurdere udviklingen af yversundhedstilstanden. Med henblik på at undersøge mulighederne for at bruge SKU, blev denne Ph.D.afhandling gennemført som en række delprojekter med følgende specifikke mål at:

- Beregne potentielt vigtige relationer mellem fremtrædende yversygdomstilstande (mælkeløb, intramammære infektioner og klinisk mastitis) og synlige karakteristika af yver og patter.
- Reducere antallet af mulige vigtige yver- og patte-karakteristika, som er relateret til yversundhed og produktion til færre typer (identificere latente yversundhedsvariable).
- Karakterisere yversundhed i besætninger med et erklæret mål om en ikke-antibiotika-behandlingspolitik og anvende disse karakteristika til at evaluere mulige negative følgevirkninger af den reducerede antibiotika anvendelse i form af ændret yversundhed.

- Give anbefalinger til anvendelsen af systematiske kliniske yverundersøgelser i den daglige styring af besætningen mht strategi for data-indsamling, relevante yver- og patte-karakteristika samt statistiske analyser.

For at nå disse mål blev der gennemført systematiske kliniske undersøgelser i tre forskellige studiepopulationer i Tyskland og Danmark. Ph.D.afhandlingen er således baseret på de følgende fire artikler:

Artikel 1 “Risikofaktorer for mælkeløb, som er relaterede til den enkelte ko”

Et longitudinalt observationelt studie blev gennemført i 15 tyske malkekvægbesætninger. Under månedlige besætningsbesøg blev mælkeflowkurverne målt under aftenmalkningen. Under den følgende malkning blev mælkeløb målt når kørerne kom ind i malkestalden. Patteform, pattespids, patteåbning og yverform hos køer i tidlig laktation (9-100 dage efter kælvning) og sen-laktation (>250 dage efter kælvning) blev målt umiddelbart efter aftagning af malkesættet. Risikofaktorer for mælkeløb blev analyseret i en logistisk regressionsmodel med besætning som random effect. Køer i første laktation med højt maksimalt mælkeflow og patteudtrækninger havde forholdsvis større risiko for mælkeløb. Høj maksimalt mælkeflow, korte patter, patteudtrækning, indadvendte pattespidser samt tidlig laktation øgede risikoen for mælkeløb hos køer fra anden laktation. Besætningsfaktoren influerede på variationen i data med cirka 10%, hvilket indikerede at betydningen af management og andre besætningsorienterede faktorer for forekomsten af mælkeløb er lav.

Artikel 2 “Ko-relaterede risikofaktorer i forhold til intramammære infektioner med *Streptococcus uberis* og *Staphylococcus aureus* hos køer i første laktation”

Et observationelt studium blev gennemført i 15 tyske malkekvægbesætninger med henblik på at identificere yver- og patte karakteristika, som var relaterede til en højere risiko for intramammære infektioner (IMI) med *Staph. aureus* og *Strep. uberis* og kliniske yverbetændelser gennem de første 100 dage af første laktation. Mælkeprøver blev sterilt udtaget ved månedlige besætningsbesøg. Yvere og patter hos alle førstekalvskøer i tidlig laktation blev vurderet umiddelbart efter mælkning. Logistisk regression med besætning som random effekt viste at afvigelse fra middelmælkeflow, vinterkælvning og patteåbningens tilstand øgede risikoen for *Strep. uberis* infektion. Risikoen for IMI med *Staph. aureus* var højere hos køer med korte, tykke patter, og i køer med spidse patter.

Signifikante risikofaktorer for klinisk mastitis var vinterkælvninger, forekomst i bagpatter, dybe yvere og store patter. Variationen i data som kunne forklares ved besætningsfaktoren i den færdige model var 2% for *Strep. uberis* infektioner, 6% for *Staph. aureus* infektioner og 16.7% for klinisk mastitis.

Dette studium indikerede at forskellige yver- og pattekarakteristika udgjorde risikofaktorer for IMI med *Strep. uberis* og *Staph. aureus* og for kliniske mastitis tilfælde.

Artikel 3 “Systematiske kliniske undersøgelser til identifikation af latente yvertyper i danske malkekvægbesætninger”

Et tværsnits-studium blev gennemført i 16 danske malkekvægbesætninger med henblik på at undersøge gennemførelsen af systematiske kliniske yverundersøgelser som et supplerende redskab til at vurdere yversundhedstilstanden. Besætningerne blev besøgt 5 gange gennem 2000 og 20 køer blev tilfældigt udvalgt i hver af besætningen under hvert besøg til undersøgelse indenfor 2 timer efter malkning. Den kliniske undersøgelse omfattede morfologiske og patologiske variable. Principal component analyse blev brugt til at trække fire forskellige yvertypologier ud, som tilsammen forklarede 30% af variationen i data: 1) det lille yver, 2) det belastede yver, 3) mastitis-yveret, og 4) det tilsvinede yver. Variable med høj positiv korrelation til “det lille yver” var lille yverform, korte patter, og første laktation. Beskadiget patte-overflade, hårdt yvervæv, og langt yver var relateret til “det belastede yver”. “Mastitis-yveret” var karakteriseret ved asymmetri mellem for- og bag-kirtler, knudret væv og akut yverbetændelse. Reduceret mælkeydelse og højt celletal var relateret til “mastitis-yveret”, hvorimod lavt SCC var relateret til “det lille yver”. “Det tilsvinede yver” var relateret til tidlig laktation. Ved at indarbejde denne information i vurderingen af yversundhed i en besætning kan være af stor værdi for data-analyser i besætninger, som mistænkes for at underrapportere forekomsten af klinisk mastitis.

Artikel 4: “Yversundhedsparametre og veterinære behandlinger i danske økologiske besætninger med en ikke-antibiotika-behandlingsstrategi”

23 økologiske malkekvægbesætninger deltog i et projekt, som havde til formål at minimere antallet af antibiotikabehandlinger. Yverne hos 50 køer per besætning blev undersøgt klinisk under malkning i begyndelsen af projektperioden. Hygiejneniveauet på krop og yver blev desuden bedømt. Data blev analyseret under anvendelse af principal component analyse og en general linear model. Principal component analysen førte til to komponenter, som kunne repræsentere to

forskellige besætnings karakteristika. Komponent 1 blev benævnt 'Snavsede, danske holstein (SDM-DH) & behandlinger', og den var karakteriseret ved yver- og kropshygiejne, årligt antal antibiotikabehandlinger af mastitis, asymmetriske kirtler og SDM-DH. Komponent 2 var derimod karakteriseret ved knuder i yveret, knudret yvervæv og trepattede køer, og blev benævnt 'kroniske yverforandringer'. Besætningens niveau mht hygiejne på baglår (i komponent 1) og prævalens af køer med knuder i yveret (i komponent 2) var associeret til det beregnede tankcelletal (CBSCC), som alene forklarede ligeså meget af variationen i data som komponenterne gjorde, og som sådan kan betragtes som en vigtig yversundheds-indikator. Antal mastitis behandlinger pr. årsko, prævalens af golde enkeltkirtler og CBSCC var ikke relateret til hinanden, hvilket indikerede at et lavt antibiotikaforbrug i sig selv ikke have en negativ indflydelse på yversundheden. Jersey-besætninger havde det laveste antibiotika-forbrug i forhold til mastitisbehandlinger, og moderat til lavt CBSCC (< 300,000). Jersey-besætningerne havde samtidig de reneste køer, men det var ikke muligt at bedømme hvorvidt disse resultater kunne tilskrives race eller management i besætningen.

Generel diskussion

I delprojekterne blev morfologiske og patologiske yver- og pattekarakteristika undersøgt i førstekalvs og ældre køer, både som individuelle variable og i form af komponenter. Adskillige yver- og pattekarakteristika viste sig at være risikofaktorer for mælkeløb, yverinfektioner som involverede forskellige bakterier, samt kliniske yverbetændelser. Denne information vil med fordel kunne inddrages i avlsprogrammer (langsigtet virkning), såvel som gårdspecifikke mastitis kontrolprogrammer (kortsigtet virkning). Principal komponent analyse var en effektiv metode til identifikation af mønstre med hensyn til yver- og pattekarakteristika, og til at kombinere med andre produktionsdata på ko- og besætningsniveau. Den kroniske yvertype blev beskrevet på besætnings- og koniveau, bestod af patologiske forandringer i yveret og var relateret til celletallet. I forhold til en ikke-antibiotika-behandlingsstrategi er antibiotikabehandlinger, forekomsten af golde enkeltkirtler og CBSCC ikke relaterede til hinanden, hvilket indikerede at en lav anvendelse af antibiotika i en besætning ikke havde en negativ effekt på yversundheden. Afgoldning af inficerede enkeltkirtler blev brugt aktivt som metode på lige fod med antibiotikabehandlinger. Forekomsten af golde enkeltkirtler må derfor fortolkes i forhold til andre yversundhedsindikatorer i vurderingen af yversundheden i en given besætning.

Som konklusion vurderes at systematiske kliniske undersøgelser af yvere bidrager til at forstå yversundhedsbegrebet, samt til at afsløre besætningsspecifikke mastitis-behandlingsmønstre samt

risikofaktorer. Systematiske kliniske undersøgelser kan bruges til evaluering og kontrol af en given tilstand, såfremt en konsistent og veldefineret udvælgelse af dyr finder sted under anvendelse af en standardiseret klinisk protokol. Indarbejdelsen af systematiske kliniske yverundersøgelser vil især være af interesse for store besætninger, i forbindelse med mistænkt underrapportering af klinisk mastitis, samt i besætninger, som ønsker at reducere antallet af mastitisbehandlinger.

1 Introduction

1.1 Background

Mastitis is the most frequent and costly disease in dairy herds (Seegers et al., 2003). Mastitis leads to economic losses in several ways. In addition to losses in milk yield and milk quality, the costs for treatment and drugs, extra labour, early culling and replacement have to be taken into account (Hogeveen and Østerås, 2005). The negative influence of mastitis on milk quality is of major concern for manufacturers and consumers (Heeschen, 1998). In recent years, there is also increasing awareness of antibiotic resistencies and residues in the milk as a consequence of high antibiotic treatment frequencies. Furthermore, mastitis can be a painful disease compromising the cow's welfare; even in cows with subclinical mastitis the concentration of pain mediators is increased (Eshraghi et al., 1999). Therefore, effective mastitis control programs are essential to meet the consumers' expectations of high standards of milk quality and animal welfare.

While there has been an overall progress in mastitis control in dairy herds in terms of reduced herd level somatic cell counts, there has been less progress in reducing the incidence rates of clinical cases of mastitis and little improvement in our ability to treat cases of mastitis and to reverse the damage done by the infection (Smith, 2005). National cattle databases and field studies show that the incidence rate of clinical mastitis per 100 cow-years at risk ranges between 20 in Norway (Østerås and Sølverød, 2005), 26 reported in a field study from the Netherlands (Barkema et al., 1999), and between 55-42 in England and Wales (Wilesmith et al., 1986). In Denmark, the incidence rate has been reported between 40 and 50 cases per 100 cow-years at risk (Bartlett et al., 2001). In many countries the incidence rate of clinical mastitis increases, at least in the Holstein population (Rupp and Boichard, 2003).

However, the use of different mastitis definitions or too general definitions makes it difficult to compare incidence rates in various studies, regional or national databases (Rupp and Boichard, 2003). Especially the true incidence of clinical mastitis is difficult to estimate, as we most likely only have access to the antibiotic treated cases of mastitis that are recorded on farms or, as in the Scandinavian countries, in national databases. Mastitis treatments and bulk milk somatic cell counts

are highly dependent on the farmer's attitude and thresholds for intervention, and cow somatic cell counts from milk recording alone will give an underestimation of clinical cases of mastitis.

With systematic clinical examinations of udders and teats (SCE), the udder health can be assessed independently of farm records. In contrast to treatment records, where we often surmise the diagnosis from the applied treatment (Dohoo and Sørensen 2000), clinical examinations reveal signs of diseases or disorders and how system and management affects the cow's udder health. Applied on herd level, clinical examinations of a representative sample of cows may identify herd specific udder health problems at an earlier stage. Several protocols for systematic clinical examinations have been developed in different countries in order to assess animal welfare or as part of a quality assurance system (Noordhuizen and Metz 2003). These clinical protocols rarely include udder health indicators. There is a need to explore the potentials of SCE as a method to compare udder health and mastitis across herds and countries, and as additional tool in mastitis control. Thorough epidemiological analysis of the different aspects of udder and teat characteristics in relation to other existing monitoring data on farm is necessary to evaluate the benefit that can be gained by implementing clinical examinations in herd health assessment.

1.2 Objective of the thesis

The aim of this Ph.D study was to identify information from systematic clinical udder examinations, which can be useful as an additional tool in udder health assessment in dairy farms. The specific objectives were to

- Estimate potentially important relations between major udder disorders (milk leakage, intramammary infections and clinical mastitis) and clinical characteristics of udder and teats.
- Reduce the large number of potentially important udder and teat characteristics related to udder health and production into fewer udder types (identify latent udder health variables).
- Characterize udder health in herds with a specified non-antibiotic treatment policy and use these characteristics to evaluate potentially negative effects of reduced use of antibiotics on udder health.

- Give recommendations for the use of systematic clinical examinations in udder health management with regard to sampling strategy, relevant udder and teat characteristics and statistical analysis.

1.2.1 Specific objectives of the subprojects

Four subprojects were carried out to achieve the objectives of the thesis.

The specific objectives were:

Subproject 1

- to examine the relation between teat characteristics and milk leakage, which is reported as being a risk factor for clinical mastitis. (Manuscript 1, Chapter 8)

Subproject 2:

- to examine the relation between udder and teat characteristics and intramammary infection and clinical mastitis in the early lactation (Manuscript 2, Chapter 9)

Subproject 3:

- to identify udder types formed by udder and teat characteristics in relation to production and inflammation parameters (Manuscript 3, Chapter 10)

Subproject 4:

- to examine udder health parameters including systematic clinical examinations in organic herds with a specified non-antibiotic treatment policy (Manuscript 4, Chapter 11)

1.3 Outline of the thesis

The thesis consists of a general part with an introduction in the subject matter, where definitions for mastitis and health as well as different methods of to assess mastitis and udder health are given. (Chapter 2). In material and methods (Chapter 3) the study design is described across the four subprojects with focus on similarities and differences between the different subprojects. Chapter 4 contains the main results of the four subprojects. In the general discussion (Chapter 5) relevant topics from all four subprojects are discussed, followed by general and specific conclusions and suggestions for future research (Chapter 6). Chapter 8-11 contain the manuscripts as the specific part with the detailed results from each subproject.

2 Literature

2.1 Pathogenesis of mastitis

Mastitis is a multifactorial disease where three biosystems are involved: the host with varying resistance to mastitis, the mastitis pathogen with differing characteristics, and the environment (Tolle et al., 1977; International Dairy Federation, IDF, 1987a). Mastitis pathogens enter and infect the mammary gland via the teat duct. Figure 1 illustrates several factors that are involved in the pathogenesis. Depending on management, genetic resistance, milking practices etc. and the condition of the immunosystem, the cow can become infected with a mastitis pathogen and develop a subclinical or clinical mastitis or remain healthy. Mastitis always begins with a subclinical stage of variable duration. In a proportion of cows the subclinical stage turns into a clinical stage. The problems related to the use of the terms subclinical and clinical will be discussed below.

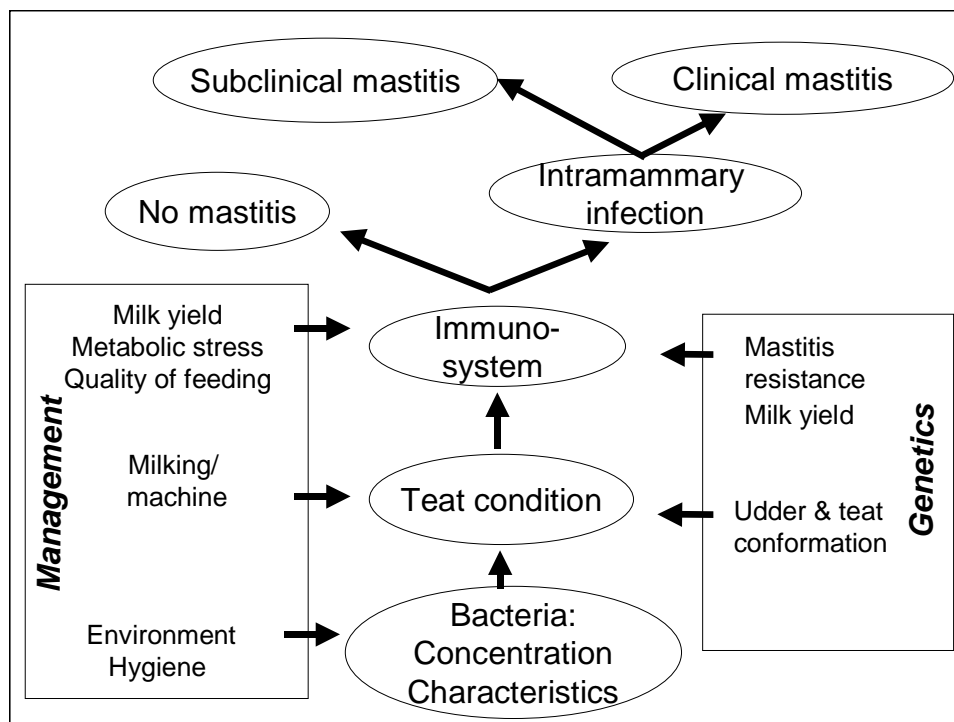


Figure 1. Simplified model to demonstrate different factors involved in the pathogenesis of mastitis
IMI = intramammary infection

2.2 Definition of mastitis and udder health

2.2.1 Treated cases of mastitis

The occurrence of clinical mastitis (CM) or incidence rates of clinical mastitis are often calculated from cases of clinical mastitis that are treated with antibiotics by the veterinarian or the farmer and are reported in farm records, veterinary treatment records or, as in the Scandinavian countries, reported to a national database. These treatments records cover the whole life span of the cows and are widely used in disease monitoring within and between farms, studies and countries. But veterinary/antibiotic treatment records are highly dependent on the farmer's decision if and when to initiate a treatment. The detection of clinical mastitis can differ widely among farmers, partly due to different management routines, e.g. foremilk procedures or control of dry cows (Bradley and Green, 2001). Vaarst et al. (2002) demonstrated a great variation of the farmers' threshold when to initiate veterinary treatment in case of clinical mastitis. Depending on the type and severity of a disease, a more or less high proportion of cows may not be detected by the farmer and/or not be treated by a veterinarian. Alternative strategies to handle mastitis cows, e.g. treatment without prescription medicine or drying off single mastitic quarters may alter the risk of mastitis and the spread of mastitis pathogens in a farm. The decision-making process whether to initiate antibiotic mastitis treatment is mainly based on four levels of choice (Vaarst et al., 2002) that were

- disease level/severity of symptoms
- cow characteristics
- herd level (replacement heifers, bulk milk cell goal, status of milk quota...)
- level of alternatives (drying off a single gland, homeopathy, peppermint oil...).

A Danish survey among 2133 farmers (Alban and Agger, 1996) has shown that about the most common practices for mastitis treatment was the application of liniment oil a.o. (60% of farmers) followed by massage and milking (34% of farmers). When the veterinarian had treated the cow with antibiotics, about 90% of the cases were reported to the Central Danish Disease Recording Scheme. The authors reported a high reliability with regard to treated cases of mastitis but still – the true incidence of CM will be underestimated. To evaluate the data quality in a Danish study on veterinary drug use including 78 herds (Bennedsgaard 2002), the farm records of disease treatments were compared to the data registered in the Danish Cattle Database (DCD). The raw use of data from the Danish Cattle Database would have lead to an underestimation of the treatment frequency of 20% in cows. Typical problems were missing registrations, wrong animal number or disease code, technical problems with data transfer, missing correction of errors, and misclassification of

diseases. The use of diseases codes may differ between veterinarians. Bennedsgaard (2002) has shown that the conversion from conventional to organic farming changed treatment patterns and treatment frequency. Research in a wider spectrum of farms, organic and conventional is necessary to enlighten the problem.

In conclusion, the incidence rates of treated cases of CM between farms reflect to a more or less extent different management strategies regarding CM (Valde et al. 2005) and cannot be used to compare the mastitis status in different herds, regions or countries.

2.2.2 Clinical mastitis

Clinical mastitis (CM) is present when any macroscopic changes in the milk and/or palpatory abnormalities of the udder are observed (IDF, 1987b). According to the severity of clinical signs and the duration of the inflammation, CM can be classified as mild or severe, acute or chronic.

Acute clinical mastitis is characterized by a sudden onset, with symptoms of inflammation such as pain, swelling, heat and abnormal milk secretion, sometimes accompanied by fever. The milk yield can be markedly reduced; severely affected cows may even stop producing milk. Mild clinical mastitis is characterised by persistent clots especially in the foremilk, while no other obvious changes in the udder can be seen (IDF, 1987b).

Chronic mastitis is characterized by its long duration with progressive development of fibrous tissue, and can be either clinical or subclinical (IDF, 1987b).

Clinical mastitis often affects individual cows in the herd and can easily be detected by inspection and palpation of the udder, typically when the farmer prepares the cow for milking.

2.2.3 Subclinical mastitis

The proportion of cows affected by subclinical mastitis is much higher and cannot be diagnosed by inspection and palpation. Subclinical mastitis can be detected by cytobacteriological examinations and is present if the somatic cell count (SCC) is elevated and a mastitis pathogen can be isolated (IDF, 1987b; German Veterinary Association, 2002). SCC is a marker of inflammation. A raise in SCC indicates inflammation and an active immune response of the affected udder quarter in order to kill the bacteria. Although extremely difficult to demonstrate, each gland of a dairy cow might undergo such a subclinical response many times a week following milking, when small amounts of bacteria may gain access to the mammary gland before the teat sphincter tightly seals the teat canal (Kehrli, Jr. and Shuster, 1994). This has to be considered when interpreting SCC and setting

thresholds for treatments, as well as physiological differences between first lactation cows and older cows, stage of lactation and breed effects (Brolund, 1986; Rupp and Boichard, 2003).

2.2.4 Healthy udder

According to the International Dairy Federation (1987b) a healthy mammary gland can only be defined by freedom from any detectable mastitis, teat canal colonization, other morbid conditions or injuries and by conformity with the common standards of normality. Equivalent healthy quarters in a mammary gland will yield similar amounts of milk of virtually identical composition.

This definition covers the element of negative health definition, i.e. if a mastitis is not diagnosed the mammary gland is classified as healthy. Whether a cow is classified as having clinical or subclinical mastitis or being free of mastitis (= healthy) depends on several factors: The applied definition of mastitis or thresholds, the methods to diagnose the different aspects of mastitis, the sensitivity and specificity of the applied methods, and the practical circumstances under which these methods are carried out. Cows with mild clinical signs such as slightly changes in the foremilk may remain undetected, depending on whether or not foremilk is practised, the light conditions in the milking parlour, the colour of the floor or the device used for foremilk, and the sensitivity of the farmer to identify changes in the milk. Hillerton (2000) reported 80% sensitivity of the farmers for detecting cows with CM during foremilk and a specificity of 100%.

The next element ‘conformity with the common standards of normality’ is highly dependent on our perception of what we regard as normal, which is based on our experience, cultural and professional background. Somatic cell count (SCC) is the most common used indicator for inflammation in the udder and measured on a continuous scale. There are different recommendations regarding the threshold of discriminating between infected and uninfected udder quarters. Griffin et al. (1987) recommend a threshold of 500,000 cells/ml to discriminate between mastitis and normal secretion of the affected quarter. The German Veterinary association defines milk from healthy quarters as being free of mastitis pathogens and having less than 100,000 cells/ml (Hamann, 2005). In composite milk samples of healthy cows the SCC also remains below 100,000. Hillerton (1999) and Smith et. al. (2001) propose a threshold of 200,000 cells/ml to discriminate between infected and

uninfected quarters and normal and abnormal milk. In Denmark no fixed threshold is proposed but the milk recording organization scores the cows' probability of having acute or chronic elevated SCC calculated on a model that considers breed, parity, stage of lactation, and course of SCC (Kjeldsen, 2000).

The most common methods of diagnosing mastitis are measurement of indicators of inflammation in the milk, identification of mastitis pathogens and inflammatory reactions of the mammary gland.

In the following paragraphs the indicators for mastitis are presented and their potentials and limitations are discussed in relation to assess mastitis and udder health in a herd.

2.3 Methods to assess udder health status based on monitoring constituents of the milk

2.3.1 Somatic cell count on quarter level

SCC is the most widely used indicator for inflammation that is used to assess udder health and milk quality on quarter, cow, and herd level (Rasmussen et al. 2005; Hamann, 2005). The California Mastitis Test (CMT) is an inexpensive and portable test to identify quarters with elevated SCC (Ersline, 2001). The inflammatory reactions are usually interpreted from a 5-point scale. Potential errors in interpretation of the results caused by person and cow effects can be reduced to neglectable levels by simultaneously testing of all 4 quarters (Enevoldsen, 1999). There is little information in literature about the use of this tool in the field. Some farmers regularly use CMT in the milking parlour to verify suspicion of changes in foremilk or to identify and monitor the quarter with elevated SCC from the monthly milk recording. Veterinarians use CMT in cows presented for treatment. Some veterinarians use CMT systematically in specific risk groups, e.g. cows before dry off, freshly calved cows (Enevoldsen, 2004). Systematic testing and record keeping is essential if results from the CMT are used in udder health monitoring.

2.3.2 Cow somatic cell counts from milk-recording

Milk recording data are often used to assess performance and udder health on dairy farms. The data are collected on a regular basis on all milking cows in the herd, and the SCC is given on cow-level.

When udder health assessment is exclusively based on cow SCC, the extent of CM (treated and untreated cases) may be underestimated. Dry cow mastitis, effectively cured mastitis between two recordings and strategies like blinding of infected quarters may not be evident in the recordings as elevated cow SCC. The genetic correlation between SCC and CM based on Scandinavian data is on average 0.7 and suggests that SCC and CM are partly the expression of the same trait (Rupp and Boichard, 2003), despite a low phenotypic correlation (around 0.3). The authors assume that based on monthly SCC only 30% of CM would be detected, i.e. those cases of CM with longer duration of elevated SCC at the following milk recording or cases of CM that occur close to the monthly milk recording.

2.3.3 Bulk milk somatic cell count (BMSCC)

The threshold for BMSCC that is regarded as acceptable for human consumption differs between countries (Hamann, 2003). In the European Union the regulatory limit for BMSCC is 400,000 cells/ml, but special quality programs pay extra premiums for milk with extra low SCC. The farmers focus on BMSCC to undergo the regulatory limit and adjust their mastitis management accordingly. Premiums motivate farmers to reduce the BMSCC further (Nightingale and Schukken, 2005; Van der Zwaag et al., 2005). In a Dutch survey, farmers were satisfied with a BMSCC of 155,000 and regarded a BMSCC of 282,000 as a serious problem (Kuiper et al., 2005). The practices commonly used to lower BMSCC are:

- Milk from affected cows is discarded
- Affected quarters are blinded
- Affected quarters are treated with antibiotics
- Non-antibiotic treatment (frequent milking, ointments, oxytocin, homeopathy)
- Dry cow therapy (selective or blanket)
- Cow culled and replaced.

Valde and colleagues (2005) found a low correlation between BMSCC and CM but a high correlation between CSCC and BMSCC. The percentage of milk delivered to the dairy was negatively correlated with nearly all studied udder health parameters except for BMSCC. Delivery % was the total amount of milk delivered to the dairy industry divided by the produced milk x 100, which was estimated from the milk recording. CSCC explained more variation of delivery % than CM indicating that farmers withhold milk from cows with elevated SCC in order to reduce BMSCC. Consequently, BMSCC is mainly an indicator for milk quality than for udder health.

2.3.4 Identification of mastitis pathogens

Quarter foremilk samples tested for SCC and mastitis pathogens are the most precise method in evaluating udder health (IDF, 1987; Hamann, 2005). Bacteriological examinations are expensive, especially because frequent sampling is necessary to detect chronic infections with *Staph. aureus* (Sears et al., 1990). A low SCC after treatment is no guarantee for cure. Mastitis pathogens such as *Staph. aureus* can remain in the udder cells and reoccur under certain circumstances. Recently, *Strep. uberis* was reported to show a similar infection pattern with chronic infections and periods of shedding (Zadoks et al., 2001). Different recommendations regarding sampling strategies can be found in the scientific literature. When all cows of the herd are tested, e.g. at yearly intervals (Nir, 1999; Hamann, 2005), the most important pathogens in a herd causing chronic infections can be detected, whereas infections with shorter duration tend to be overlooked (Enevoldsen, 1999). The culturing of all clinical cases of mastitis is an efficient strategy to determine the distribution and dynamics of mastitis pathogens with short duration (Andersen et al., 1996; Enevoldsen, 1999), to determine whether an antibiotic treatment is justified and to choose the appropriate drug (Roberson, 2003). In order to assess the importance of mastitis pathogens cultured from cases of CM and to monitor their development within and across herds, the criteria for selecting mastitis cases for culturing have to be well defined and recorded. Especially in larger herds with different milking staff the mastitis treatment plan has to be written down to ensure that all involved in cow management select and treat cows after the plan (Roberson, 2003).

2.3.5 Other indicators

Electrical conductivity (EC) is increased in mastitis milk because of its higher content of sodium and chloride ions (Erskine, 2001). The highest sensitivities and specificities are achieved when EC is measured on quarter level of foremilk and interquarter differences are used to identify cows with elevated EC. Hand-held conductivity meters can be used as cow side screening test similar to the CMT. On-line milk meters that can measure EC on cow level are commonly installed in modern milking parlours. In automatic milking systems (AMS) EC is measured on quarter level and used for udder health monitoring. Cows with higher than expected EC are flagged on alarm lists for visual control. Technical equipment to measure EC, software and algorithm to identify cows at risk of having mastitis differ between AMS models. The sensitivity and specificity vary across herds and AMS models and currently the sensitivities are too low for automatic sorting of abnormal milk (Rasmussen, 2005). In order to compare udder health across herds, EC measurements should be

standardized. PH indicator paper is a cow side test to detect mastitic quarters and often used as alternative to CMT. Like with the CMT, abnormal quarters are mainly detected by inter-quarter comparison (Sandholm, 1995). Other inflammatory parameters in the milk like NAGase, lactose or lactate are expected to become diagnostic tools in commercial dairy farms in the future (Hamann, 2005).

2.4 Systematic clinical examinations in udder health assessment

Because of all the negative effects of mastitis, the assessment and monitoring of udder health is crucial for an efficient management in dairy herds. As demonstrated above, single indicators for mastitis are not sufficient to monitor udder health and to analyse the situation. When combining information from different sources, e.g. CSCC, treated cases of clinical mastitis and BMSCC, the effect of the farmer's management on the indicators still remains. Indicators may not be applied systematically, e.g. CMT-scores and milk samples for culturing could be sampled irregularly in special cases only. When CMT or milk samples are collected systematically, e.g. all cows before drying off and in early lactation, the recorded results may be mixed with those collected for other reasons without stating the sampling criteria. Criteria or management practices regarding mastitis treatments may vary as well as the quality of record keeping. Consequently, a standardized method to assess and monitor the development of udder health is needed for several reasons. 1) Udder health can only be compared across herds with a standardized method, which is independent from the farmer's management and record keeping. 2) Within a herd, a standardized method is necessary in order to assess the effect of changes in management on udder health. Systematic clinical examinations (SCE) including udder and teat characteristics may fill this gap.

2.4.1 Definition of Systematic Clinical Examinations

Systematic clinical examination is the clinical examination of unambiguously defined samples of cows following a standardized clinical protocol with the aim to document the health situation within or across herds, to identify health related problems and the related risk factors, and to monitor the development of the health situation.

Sampling strategy

There are two general approaches of selecting cows for SCE: 1) random sampling of cows that are representative for the study population or 2) sampling of all cows within specified risk groups.

Random sampling means that cows are randomly selected regardless of parity, stage of lactation or breed. The aim is to obtain an udder health status representative for the entire herd at a specific point of time: to identify udder health problems and related risk factors within the herd, herd specific patterns and groups of cows with higher risks for certain clinical conditions. SCE of a random sample can be carried out once to have an udder health status as decision support or at regular intervals to monitor the development of the clinical conditions of interest. Random sampling of a representative group can as well be applied to document udder health status as a part of a certification or quality assurance program and in welfare assessment.

The second approach is to sample specified high-risk groups, e.g. cows in specific stages of lactation or parities. This implies that high-risk groups are already identified, e.g. as a result of a SCE of a random sample. These high-risk groups are regarded as early indicators for emerging udder health problems. Typical high-risk groups can be cows in specific stages of lactation, e.g. in early lactation, at peak lactation or before drying off. The major advantage of this approach is the reduction in sample size per SCE. If the clinical condition of interest only occurs at a specific stage of lactation, e.g. periparturient udder oedema, the second approach is the only feasible. With frequent sampling of high-risk groups, e.g. at weekly or monthly intervals, the udder health state of the high-risk groups is monitored. The sampling interval depends on the type of the high-risk group, herd size, calving patterns and the clinical conditions of interest. Both sampling approaches aim at determining prevalences of udder health disorders and diseases. According to the expected prevalences and thresholds for decision-making the appropriate sample should be selected.

In conclusion, sampling criteria have to be well defined and sampling has to be carried out consistently. The clinical protocol has to be standardized, and the clinical examination has to be carried out in the same manner in each herd and at each visit. Only results that are gathered according to the criteria stated above can be used to assess the udder health status within and across herds, to analyse related risk factors and to evaluate the development over time.

2.4.2 Clinical udder examination

The clinical udder examination can be divided in a general part that includes the general condition of the cow (the posture, behaviour, body condition, body temperature, respiratory rate, pulse frequency, and rumen motility) in order to assess if or how much the general health of the cow is affected, and a special part, where udder and milk are assessed (Sandholm and Pyörälä, 1995). The specific examination of the udder includes inspection and palpation of udder and teats and the assessment of milk secretion (Grunert, 1990). Traditionally, the veterinary practitioner focuses mainly on acute (severe) clinical mastitis and uses the obtained information of the general and udder examination to give a precise diagnosis and prognosis about cure and future use of the individual affected cow.

In SCE the interest of clinical examination is to describe udder health in a group of cows and to reveal the cows' response to management, system and environment. The SCE consists of many variables describing different aspects of udder and teat health that form a cow and herd specific pattern. Pattern recognition is a very efficient strategy to solve clinical problems, and leads to a successful diagnosis when cases are recognized as being similar to one seen before (Corderre et al., 2003). In order to recognize patterns, multiple examples must be in memory. The examiner or clinician has to see (in terms of 'to notice') and assess all clinical signs displayed by the SCE, and interpret them to give the most probable cow and herd diagnosis and to propose a herd specific mastitis control plan.

The information obtained by the clinical udder examination derives from three focus areas: morphologic features (traits) of udders and teats, milking machine/milking related or environmental related changes of udders and teats that can be physiological or pathological (trauma, lesions), and pathologic changes in udders and teats caused by infections. Table 2.1 lists the examined clinical udder and teat characteristics reported in the literature.

Table 2.1. Overview over udder and teat characteristics within the focus areas morphology, milking related/environmental changes and pathologic changes.

Location	Morphologic features (traits)	Milking related/ Environmental changes	Pathologic changes caused by infection
Udder	Shape Position Attachment Udder-floor distance	Asymmetry of quarters Skin condition	Asymmetry of quarters, Atrophy of quarters, Swelling, hardness of udder tissue Lumps, nodes
Teats	Shape (diameter/length) Position Shape of teat end	Skin condition Teat end condition (Hyperkeratinization) Swelling Discoloration	Viral infections Bacterial infections Parasitic infections

2.4.3 Morphologic features (traits)

Udder and teat conformation and their relations to high SCC and CM are widely used in breeding programs to select cows with better genetic resistance to mastitis. Especially in the Nordic countries that have national disease recording databases, the relationship between conformation and CM defined as cases of veterinary treated mastitis has been studied. High and strong udder attachment result in less clinical mastitis (Slettbakk et al., 1995) and lower SCC (Seykora and McDaniel, 1985; Ronningen and Reitan, 1990; Rupp and Boichard, 2003). The reports on the relationship between teat length and mastitis are discussed inconsistent. Longer teats were positively correlated with CM (Lund et al., 1994), whereas Slettbakk et al. (1995) and Neijenhuis et al. (2001b) could not find an association between teat length and the risk of CM. Lund et al. (1994) report a very low genetic correlation (0.01) between teat length and SCC in contrast to others who reported genetic correlations of 0.16-0.20 (Seykora and McDaniel, 1985; Rogers et al., 1991). These different findings could partly be explained by the interaction between liner and teat during milking. Too short teats may not reach the collapsing point of the liner during milking, which will result in a lack of massage and increase the risk of intramammary infection (Rasmussen et al. 1998 and 2004). When teats are very long, they will penetrate the liner beneath the collapsing point, which is also associated with an increased infection rate (Ronningen and Reitan, 1990; Rasmussen et al. 1998). Slettbakk et al. (1995) found borderline effects of wide teat diameters and the risk for clinical mastitis. Funnel shaped teats have been reported to have a lower frequency of mastitis and a lower SCC (Rathore, 1976). Cows with inverted or pointed teat ends had higher SCC than cows with normal teat-end shapes (Slettbakk et al., 1990).

2.4.4 Milking related and environmental changes

Poor milking management, deficiencies in the milking machine function and the environment can have negative effects on teat and udder health and increase the risk for intramammary infections.

Teat condition can undergo short-term changes as response to a single milking, e.g. the color, firmness, thickness or swelling of teats and the openness of the teat orifice (Mein et al., 2001). The type and intensity of a change in the teat condition and the number of cows affected in the herd indicates the type of malfunction of the milking equipment or milking management. Medium-term changes are visible after a few days or weeks and include dry, chapped or cracked teat skin (cold, wet, muddy weather conditions, type of teat dip), and vascular damage (pulsation failure, high vacuum, overmilking) (IDF, 1987c; Mein et al., 2001). Often multiple machine-related factors have to be suboptimal to increase the risk for mastitis. Overmilking alone did not affect SCC in experimental farms (Natzke et al., 1978) or the number of cows with new infection (Natzke et al., 1982), but increased the risk of cross-contamination within cows (Natzke and Bray, 1985). Under field conditions, Osteraas and Lund (1988) observed a higher risk for subclinical mastitis in overmilked cows, which may have been caused by irregular vacuum, air admission and impact particles.

The changes of the degree of teat end callosities are long-term effects (Mein et al., 2001). Machine milking stimulates a moderate keratinization of the epithelium in the teat canal and is a physiological reaction. A higher degree of keratinisation is called hyperkeratinisation and is often seen as white smooth or rough calluses at the teat orifice. Over-milking or pulsation failure can increase the degree of teat end callosity (Neijenhuis et al., 2001a). The degree of teat end callosity is related to clinical mastitis. Cows with clinical mastitis during the second until the fifth month of lactation were found to have a higher degree of teat end callosity in comparison to cows without clinical mastitis (Neijenhuis et al., 2001b). The milking management can affect the udder tissue. The positioning of the milking unit influences the degree of residual milk after detachment. As a consequence udder quarters may become asymmetric.

2.4.5 Pathologic changes caused by infections

Swelling, redness, pain and heat are the typical signs of an udder quarter with acute clinical mastitis. After the acute clinical stage, quarters may heal without leaving any visible or palpable signs (*restitutio ad integrum*) (Schulz, 1994) or they may become chronic. Typical signs of a chronic infection are lumps in the quarter, fibrous tissue, atrophy or involution of the gland,

firmness of parts or the complete glandular tissue. Milk secretion can be decreased. The quarter can change between acute inflammation, reburst of inflammation or chronic subclinical inflammation.

Inflammations in the teat skin are usually caused by viruses, bacteria, seldom by fungi and parasites (Hillerton et al., 2001). The infections can cause primary lesions or be secondary infections after initial trauma caused by milking machine deficiencies or environmental damage.

Often the line between physiological and pathological is not quite clear, e.g. periparturient oedema is physiological but an intensive oedema is regarded as pathological and increases the risk of getting CM (Slettbakk et al., 1995).

2.4.6 How do systematic clinical examinations support udder health monitoring?

Most studies focus on clinical features within 'their' discipline. Currently morphologic variables are mainly used for breeding purposes and not collected systematically in larger groups of cows in a herd. SCE in terms of milking related changes are recommended for problem herds, spreadsheets for recording are offered by some extension services and universities. Because mastitis is a complex and multifactorial disease, it has to be dealt with in an interdisciplinary approach by combining udder health information from the focus areas anatomy, milking related changes, and pathologic changes. With standardized definitions of the clinical variables, the results of SCE can be used to detect changes in udder and teats at an early stage, i.e. before a mastitis problem arises. Furthermore, the development of the udder health status within a herd can be monitored and udder health can be compared between herds (bench-marking).

The potential of the different approaches of applying SCE has to be investigated. SCE can be carried out as cross-sectional examination of all cows within a herd to obtain a detailed udder health description of all at a certain point of time. System-related risk factors and high risk-groups can be identified. Another approach is to carry out SCE of high-risk groups within a farm, e.g. cows before drying off and in early lactation. This approach is especially feasible in herds that already apply a herd health program with systematic clinical examinations and recordings, e.g. systematic body condition scoring of all cows at dry off, calving and during the breeding period or systematic examination of the reproductive system of all cows at calving and in the breeding period (Nir, 2004). In order to evaluate the potentials and limits of implementing SCE in a herd health program, the different approaches of SCE have to be investigated in a variety of typical modern farming systems.

3 Material and Methods

3.1 Study populations and study design

The observational data analysed in manuscript 1 – 4 were collected in three different study populations in Germany and in Denmark during three different time periods and research projects. Herd characteristics and study design are shown in Table 3.1.

The data for manuscript 1 and 2 were collected within a project to improve udder health in loose housing systems in Schleswig-Holstein, Germany (1997-1999). The selected herds participated in the milk recording system, had loose housing systems, represented the three typical local breeds and had their milking plant annually checked. The farms were visited monthly during the morning milking, except for July 1997 and July 1998.

Manuscript 3 ‘udder types’ is based on data collected from the CEPROS-project, ‘The influence of production facilities, management strategies and medication policy on health and antimicrobial resistance in dairy cattle herds’ (1999-2002). A clinical protocol for udder examination was developed and applied in 16 farms. The farms were located in the Kongeå region in Southern Jutland, Denmark, and were conventional farms with loose housing or tie stall housing.

The data analysed in manuscript 4 were collected in 23 organic herds delivering milk to the Thise dairy in Denmark, which is approximately 50% of the Thise Dairy’s milk producers. The farmers participated in the innovation project ‘Phasing out antibiotics in organic farms’ (2004-2006). The farms were visited once in March/April 2004 during the morning or evening milking.

All study farms were commercial farms, which was one important reason for aiming at minimum interference with normal daily milking routines. First, it was the best way to ensure the compliance of the farmers in the project, and secondly, for later implementation of SCE into practice it was necessary to develop and apply protocols that are feasible under practical farming conditions. Increasing the normal milking duration with a maximum of 30 minutes per farm visit was regarded as acceptable. Therefore, not all cows within a herd could be examined during one milking and a

sample size had to be chosen according to the purpose of the respective study. Two different strategies of selecting cows for clinical examination were used. In manuscript 1 and 2 cows were selected at specific stages of lactation that were regarded as high risk periods, i.e. cows from calving to 100 days in milk (DIM) and cows with more than 250 DIM. In manuscript 3 and 4 cows were selected randomly. Before the beginning of milking, the milking stalls were determined with regard to the required sample size and number of groups to be milked, e.g. only cows standing in stall 1, 3, 5 and 7 at each side of the milking parlour or every other stall in a rotary parlour was chosen to achieve a random distribution of cows with regard to rank, stage of lactation, and health state.

Table 3.1. Study design and herd characteristics

	Manuscript 1 'milk leakage'	Manuscript 2 'IMI primiparous cows'	Manuscript 2 'udder types'	Manuscript 4 'non-antibiotics- policy'
Country and region	Germany, Schleswig- Holstein	Germany, Schleswig- Holstein	Denmark, Southern Jutland	Denmark, Mid and Northern Jutland
Year	Jan. 1997-Dec. 1998	Sep. 1997-Dec. 1998	2000	2004
No. of herds	15	15	16	22
No. of farm visits	22, monthly	14, monthly	5, bimonthly	1
Examiner	Author	Author	2 veterinarians, 2 research technicians	Author
Sample size	All cows < 80 and > 250 DIM ¹	First lactation cows < 100 DIM	20 randomly chosen cows/visit	60 randomly chosen cows
Time and place of	During milking	During milking	Within 2 hours after milking	During milking
Herd size	75	75	87	73
Breed	German Holsteins		Danish Holsteins	Danish Holsteins, Jersey
Housing type ²	FS	FS	Tied, FS, SY	FS, SY
Type of production	Conventional (1 organic)	Conventional (1 organic)	Conventional	Organic
ECM 305 ³	7753	7695	7753	7176
CBMSCC ⁴	178	299	178	250

¹Days in milk

²FS = free stall housing with concrete or slatted floor, SY = straw yards

³Energy corrected 305 day milk in kg

⁴calculated bulk SCC

3.2 Data Sources

3.2.1 Monthly milk recording

In all manuscripts data from the milk recording were used. On monthly farm visits milk samples are taken from all lactating cows of the herd. The samples were analysed and the 24 h milk yield in kg, milk fat and milk protein percent, and the CSCC are determined. In Germany the control organization of the federal state collects the mandatory data on herd identification, cow identification, herd of birth, date of birth, movement and slaughter. Furthermore data on calving, calving ease, and culling reasons are collected. In Denmark all data from milk yield control as described above is gathered in the national Danish Cattle Database.

3.2.2 Mastitis treatments

In comparison to Germany, the use of veterinary drugs is more strictly regulated in Denmark. The treatment of cows with hormones, antibiotics, analgesics and anthelmintics requires a veterinary diagnosis. The owner can carry out follow-up treatments if he/she has signed a herd health advisory contract with the veterinarian with monthly herd visits. Prophylactic use of antibiotics is not allowed, which implies that blanket dry cow treatments are prohibited. Dry cow therapy can be carried out if the veterinarian has diagnosed mastitis on quarter level, a bacteriological culture is positive or if the cow has a registered mastitis 30 days prior to drying off (Anon. 2002a and 2002b). Veterinarians and farmers report diseases treatments to the Danish Cattle Database. Data on veterinary mastitis treatments from the Danish Cattle Database were used in manuscript 4.

In Germany blanket dry cow therapy is a common practice and was applied in all studied herds (manuscript 1 and 2). During the study period in 1997 and 1998 all mastitis treatments, carried out by the veterinarian or by the farmer, were registered by the farmer on record cards or in the herd management system of the farm. The author collected the data on monthly farm visits.

3.3 Systematic clinical udder examinations

In manuscript 3, two veterinarians and two experienced and specially trained research technicians performed the clinical examinations. After the first visits to each farm, the results were discussed to increase inter- and intra-observer agreement and the recording sheet was adjusted. The research technicians performed the clinical examinations during the subsequent farm visits. The cows were examined within two hours after milking. The clinical examination was conducted based on the

method described in Rosenberger (1979) and further developed by Houe et al. (2002). In addition to udder and teat characteristics, the degree of soiling with manure of thigh, udder and teats was recorded (Table 2.2). All quarters of a cow were examined. If the degree of a condition differed between quarters within a cow, the most critical condition was recorded (e.g. if poor skin quality was observed on one teat, this was the recorded result from this cow). Because of the adjustments that were made in the recording scheme after the first farm visit, records of the first farm visit were omitted from further analysis. Table 3.2 gives an overview over the clinical variables analysed in the different manuscripts.

Table 3.2. Clinical variables analysed in manuscript 1-4.

Variable	Manuscript 1 'milk leakage'	Manuscript 2 'IMI primiparous cows'	Manuscript 3 'udder types'	Manuscript 4 'non-antibiotics- policy'
Asymmetry between hind quarters/ Asymmetry between front quarters			x	x
Dry quarter				x
Clinical mastitis			x	
Udder edema	x	x	x	
Knotty tissue			x	x
Distinct nodes				x
Udder tissue condition			x	
Udder depth	x	x		
Udder shape			x	
Teat shape	x	x	x	
Teat end shape	x	x		
Teat wound			x	
Scar tissue in teat canal			x	
Skin quality of teats			x	
Warts on teats			x	
Warts on top of teats			x	
Wounds on warts			x	
Teat end callosity	x	x	x	
Soiling on teats/udder			x	x

The SCE in manuscripts 1, 2 and 4 were carried out by the author in the milking parlour. Udders and teats were inspected and palpated immediately after detachment of the udder. The presence or absence of milk leakage in manuscript 1 was observed while the cows entered the milking parlour. The clinical examinations in manuscript 1 and 2 were mainly based on Rosenberger (1979) and Grunert (1990). The clinical examinations in manuscript 4 were based on experiences from

manuscripts 1 to 3. The variable ‘dry-quarter’ was included in manuscript 4, because it was expected that farmers might dry off udder quarters with mastitis as alternative to antibiotic treatment. To have a more detailed description of pathologic changes in the udder tissue, pathologic changes were recorded at knotty tissue and distinct nodes in manuscript 4.

In two farms in manuscript 4, cows were milked in an automatic milking system (AMS). One farm had cubicle housing and forced cow traffic. The cows were examined in the cubicles. The other farm had a deep litter system. SCE was carried out during milking in the automatic milking unit (inspection) and immediately after unit detachment (inspection and palpation).

3.4 Statistical analysis

The statistical methods applied to analyse the collected data were, linear regression, logistic regression with random effects and principal component analysis. Table 3.3 gives an overview.

3.4.1 Logistic regression with random effects

Logistic regression was used to assess risk factors for milk leakage in manuscript 1 and risk factors for intramammary infections and CM in manuscript 2. The collected data in manuscript 1 and 2 had a hierarchical structure showing the clustering: quarter within cow and cow within herd. For data with binary outcome a marginal logistic regression model (generalized estimation equations mode, GEE) can be applied in SAS (Proc genmod). The GEE accounts for the correlation structure between the clusters, but gives only the estimates and tests for the fixed effects. Mixed models that include random and fixed effects estimate the fixed effects and the variance components of the random effects. Therefore the Glimmix macro in SAS was used to analyse the binomial data and to assess the random effects (Littell et al., 1996).

The clinical variables in manuscript 1 were collected on quarter level and the initial hierarchical structure was: herds, cows within herd and quarters within cow. These models resulted in serious under-dispersion. The dispersion parameters for the different models were in the interval 0.6-0.13. The dispersion parameter allows the residual variances to deviate from their expected values. A value greater than one indicates more variation than expected by chance and is referred to as over-dispersion.

Table 3.3. Statistical techniques applied in the 4 subprojects

	Manuscript 1 'milk leakage'	Manuscript 2 'IMI primiparous cows'	Manuscript 3 'udder types'	Manuscript 4 'non-antibiotics-policy'
Regression model	Milk yield modelled, residuals used to characterise cow as low/average/high/yielder in comparison to other cows within the same parity		Milk yield modelled, residuals used to characterise cow as low/average/high/yielder in comparison to other cows within the same parity	Influence of udder health parameters to cBSCC - individual variables - principal components
Logistic regression with herd as random effect	Risk factors for milk leakage; Residuals from milk yield model used as risk factor; 1 quarter of a cow from 1 observation selected randomly	Risk factors for IMI with <i>Staph. aureus</i> , <i>Strep. uberis</i> and CM 1 quarter selected: - randomly in healthy cows - affected quarter in affected cows		
Principal component analysis (PCA)			- stepwise included: 1. clinical variables 2. parity, stage of lactation 3. SCC, results from milk yield model -cow level variables	clinical variables, production data and other udder health parameters included -herd level variables

Similarly, a value less than one indicates less than expected variation and is referred to as under-dispersion (Brown and Prescott, 1999). A solution to this problem could have been to aggregate quarter observations on cow level, because only 9 cows out of 131 with milk leakage had less than four affected quarters. Because the levels in the categorical variables could not be regarded as linear, an aggregation on cow level as a cow-median or cow-mean was not reasonable. Therefore,

one quarter per cow was selected randomly. This approach still permitted evaluation of quarter level risk factors. The model building process continued with herd as a random effect.

In the initial analyses in manuscript 2 all data from all quarters were included and cow specified as a random effect. This resulted in serious under-dispersion because there was very little variation between the quarters of a cow with regard to teat shape, teat end shape and teat orifice level. The solution chosen in manuscript 2 was to select one quarter per cow and to use one observation per cow because this approach permitted us to evaluate quarter level risk factors. In cows where one quarter was affected with IMI or CM, the affected quarter was chosen for analysis in the model. In cows where more than one quarter was affected with IMI or CM, one of the affected quarters/cow was chosen at random. In the healthy group, one quarter/cow was chosen at random. The model building process continued with herd as a random effect.

3.4.2 Principal component analysis

The various indicators chosen to describe udder health may be closely related to each other and they may be insufficient to describe udder health status as single variables. Principal component analysis (PCA) is a technique that removes multicollinearity and provides insight into how the variables are related to each other (Dohoo et al. 1996 and 2003). The information contained in all predictor variables is consolidated into a set of uncorrelated predictor variables (= components). PCA attempts to identify a minimum number of components explaining the majority of the variation in the original data.

Principal component analysis was performed in manuscript 3 and 4 using the factor procedure of SAS (Proc factor, SAS, 2000). The number of components to retain was determined by evaluating the eigenvalue-one criterion, the scree test, the proportion of variance accounted for and the interpretability criteria (Hatcher, 1994). Because each observed variable contributes one unit of variance to the total variance in the data set, a component displaying eigenvalues greater than one accounts for a greater amount of variance than one variable alone and is worthy to be retained.

Further, a component was regarded as important and kept for further analysis if at least three variables had high loadings on that component and shared the same conceptual meaning. A factor loading greater than +/- 0.35 was considered to be important.

A loading represents the correlation of a given variable with the underlying factor. Loadings range from -1 to $+1$. Principal component analysis (PCA) attempts to identify a minimum number of components, explaining the majority of the variation in the original data. The first component extracted in a PCA accounts for a maximal amount of total variance in the observed variables (Hatcher, 1994). Typically, the second component will be correlated with some of the observed variables that did not display strong correlations with the first component. Further, the second component is uncorrelated to the first component. The successive components identified explain progressively smaller portions of the total sample variance. Linear combinations of observed variables are formed. In contrast to common factor analysis, in PCA no assumptions about the underlying common parts (communalities) and unique parts of the variables are made (Rummel, 1970).

Manuscript 3 ‘udder types’

All variables were analysed on individual cow-level. The scree test displayed four components with the largest eigenvalues before a visible ‘break’. Because ordinary PCA does not account for the effect of the individual cow, only the last observation per cow was included in the analysis ($n = 707$). Therefore, only clinical examinations conducted by two technicians were analysed. PCA was carried out on cow-level with categorical and continuous variables. Udder shape, teat shape, parity and stage of lactation were treated as dummy variables with normal udder and teat shape, second parity and middle part of lactation as reference level. In cases of these dichotomous variables, the loadings on a factor were interpreted as the probability of its presence (positive loading) or absence (negative loading), given that the factor exists (Rummel, 1970). Three different PCAs were performed: First (PCA 1), only clinical variables were included in the analysis to simulate the situation of the practitioner on farm with no further information about the cows. Second (PCA 2), the variables parity and stage of lactation were added as dichotomous variables. Third (PCA 3), milk yield and previous SCC were added. The non-orthogonal promax rotation with a power of 3 was used to enhance the interpretability of the factors.

Manuscript 4 ‘non-antibiotics-policy’

PCA was carried out with herd-level variables. According to the criteria stated above, 2 components were retained for further analysis in manuscript 4. The relation between the extracted components with the CBSCC was analyzed with a general linear regression model (proc GLM, SAS 8.2).

3.4.3 Modelling of the lactation curve

In manuscript 1 and 3 milk yield was adjusted for parity group and stage of lactation to control for multicollinearity and to be able to characterize a cow as high or low yielding cow in comparison to other cows in the same parity group and stage of lactation. The energy corrected total milk yield from the official test-day of all cows in the herds was used to model lactation curves. By applying a piecewise-linear model, a lactation curve for three parity groups was fitted (parity 1, 2 and >2). The method was suggested by Enevoldsen et al. (2000) and applied by Nielsen et al. (2002).

When analysing ECM from test day records to model the lactation curve, the change in slope was expected to occur around 60 days after calving (Enevoldsen et al., 2000). A standard lactation milk curve of the current lactation was estimated for each cow by using a piecewise linear regression model (Proc mixed, SAS, 2000) as described by Enevoldsen et al. (2000). The peak of the lactation curve was set to day 60 after calving. It was the intercept of the model and was set to zero (DIM60):

$$\text{DIM60} = (\text{DIM}-60)/245 \quad [1]$$

The second variable (DIMB60) was defined, which has the value 1 if DIM is below 60, otherwise 0. This variable models the slope from calving until 60 DIM:

$$\text{DIMB60} = \text{DIM60}(\text{DIM} < 60) \quad [2]$$

The model accounted for the effect of parity and stage of lactation and was

$$Y_{ijk} = \mu + \text{Parity}_i + \text{DIM60}(\text{parity})_j + \text{DIMB60}(\text{parity})_k + \varepsilon_{ijk} \quad [3]$$

Where

Y_{ijk} = estimated ECM

μ = expected mean at 60 DIM among third parity cows

Parity_i = fixed effect of parity ($i = 1, 2$, and $3+$)

$\text{DIM60}(\text{parity})_j$ = fixed effect of DIM within parity

$\text{DIMB60}(\text{parity})_k$ = fixed effect of ‘before or after day 60’ within parity.

The intercept is an estimate of the peak milk production at day 60 after calving and was 32.4 kg for cows in the third parity group in manuscript 3. The estimate of DIM60(parity) is the estimated difference in milk production between exactly 60 DIM and 305 DIM within the different parity groups. Cows in first lactation had the lowest difference of -4.1 kg ECM between DIM 60 and DIM 305 indicating the highest persistency. The estimated difference in milk yield between the day of calving and 60 DIM in second parity cows was not significant, indicating a falling line from calving until day 305 instead of a peak at 60 DIM, which is common in models of milk yield where milk is not adjusted to energy corrected milk.

The normally distributed residuals of the model represent the parity-specific deviation of the expected milk yield from the measured milk at any point during lactation according to the lactation curve of all cows in a specific parity. The mean residual of the complete lactation for each cow was used as a continuous variable for further analysis. In manuscript 1, the mean residuals entered the random effect logistic regression model with milk leakage as binary outcome as risk factors. In manuscript 3, the means residuals entered PCA 3.

A cow's mean residual derived from the piecewise-linear model characterized the cow as yielding higher, lower or as expected in comparison to all cows in that parity across herds. The mean residuals of the cows included in the PCA 3 ranged from -15.0 to 16.1 kg ECM below or above the expected ECM, the mean was 0.50 ± 4.3 kg.

4 Results

4.1 Cow-level risk factors for milk leakage

Milk leakage was observed in 5.3% of the cows, in 6.1% of the primiparous cows and in 4.8% of the multiparous cows. In the multiparous cows, the average peak milk flow decreased from 3.7 kg/min in the first month of lactation to 3.0 kg/min > 250 DIM.

Primiparous cows with higher peak milk flow than the average in the same stage of lactation had a higher risk of milk leakage. Further, cows with teat canal protrusions were at higher risk of milk leakage in comparison to normal teat orifices as well as in comparison to all other orifice characteristics. The lower yielding cows and the cows in early lactation were at slightly higher risk of milk leakage. Teat shape, teat end shape, edema, quarter position and breed did not affect the risk of milk leakage in primiparous cows. The variation between cows within herd accounted for 89.2 % of the total variation.

The multiparous cows with higher peak milk flow than the average in the same stage of lactation were at higher risk of milk leakage. The risk of milk leakage was higher in the beginning of lactation. Further, the risk of milk leakage was increased in cows with teat canal protrusion, inverted teat end shape and short teats. Milk yield did not affect the risk of milk leakage in multiparous cows. Quarter position was of borderline significance. The cow level variance component was 89.6%.

4.2 Risk Factors for Intramammary Infections (IMI) with *Strep. uberis* and *Staph. aureus* and Clinical Mastitis (CM) in First Lactation Cows

4.2.1 IMI with *Strep. uberis*

Deviation from the peak milk flow (DevPMF) was a significant risk factor for IMI with *Strep. uberis* (Table 6). The mean DevPMF for healthy cows was 0.04 ± 0.86 kg/min whereas cows with *Strep. uberis*-infection had a mean DevPMF of 0.45 ± 0.93 kg/min. Calving in winter and teat canal

protrusion as well as rough teat end callosity were significant risk factors for IMI with *Strep. uberis*. The random herd effect was low and explained 2 % of the variance. When herd prevalence of *Strep. uberis* entered the model, the random herd effect dropped to zero. There was a linear relationship between herd prevalence of *Strep. uberis* and the risk of infection with *Strep. uberis*.

4.2.2 IMI with *Staph. aureus*

The herd prevalence was the most important risk factor for IMI with *Staph. aureus*. The relationship was non-linear and showed a linear increase up to a prevalence of 2% and a curvilinear slope with higher herd prevalences. When herd prevalence entered the model, the random herd effect decreased from 29.4% to 6%. Cows with short and thick teats as well as cows with pointed teats were at higher risk of infection with *Staph. aureus*.

4.2.3 Clinical mastitis

From calving to DIM100 52 primiparous cows experienced at least one episode of clinical mastitis. Clinical mastitis was not observed among the 23 cows with small and thin teats and in four cows with pointed teat ends. Cows with udders at hock level and long and thick teats were at higher risk of clinical mastitis. There was confounding present between milk leakage and calving in winter. Cows calving during the winter season were more likely to have milk leakage than cows calving during the summer months. The herd prevalence of *Strep. uberis* was a significant risk factor in the univariable model, but confounding was observed with calving season. Because management during calving season influences the infections risks and herd prevalence, calving season was kept in the model process. The random herd effect accounted for 16.7% of the variance in the data.

4.3 Systematic Clinical Examinations for Identification of Latent Udder Health Types

The principal component analysis (PCA) extracted four components explaining 27% of the variability of the data (Table 4.1): 1) the small udder, 2) the distressed udder, 3) the mastitis udder, and 4) the soiled udder. The first component described the “small udder” in contrast to the deep udder. Variables with the highest positive loadings on the component were first parity, small udder and short teats. Variables with high negative loadings were parity 3 +, deep udder and MLOGSCC. The second component was labelled “distressed udder” because variables describing an impaired

teat surface such as wounds on warts, poor skin quality and teat wounds were loading high on the component. Further, a long or backwards bulging udder and fleshy teats were related to the component. The third component was labelled “mastitis udder” because it consisted of variables describing acute alterations (clinical mastitis) and chronic alterations (asymmetry, knotty tissue) of the udder. Cows loading high on the “mastitis udder” had a higher MLOGSCC as well as a lower MRESMILK. The fourth component was labelled “soiled udder”. Cows loading high on “soiled udder” had soiled udder and teats and were more likely to be in early lactation than in other stages of lactation.

Table 4.1 PCA 3: Significant correlations (*correlation coefficients > 0.35) between udder health parameters, parity, stage of lactation, expected milk yield, SCC and 4 components after oblique rotation (promax rotation, power=3).

Variables	Component 1 Small udder	Component 2 Distressed udder	Component 3 Mastitis udder	Component 4 Soiled udder
First parity	0.77*	0.01	-0.07	-0.06
Small udder	0.63*	0.06	0.04	0.19
Short teats	0.39*	0.06	0.19	0.08
Deep udder	-0.46*	0.04	0.13	0.12
Parity 3 +	-0.76*	0.08	0.07	0.02
Wounds on warts	0.09	0.63*	-0.11	-0.07
Udder hardness	0.12	0.57*	0.25	0.02
Skin quality of teat	-0.18	0.56*	0.06	0.14
Long udder	-0.04	0.39*	-0.23	-0.13
Asymmetry between hindquarters	0.01	-0.04	0.46*	0.13
MLOGSCC ²	-0.35*	0.08	0.45*	-0.01
Asymmetry between front quarters	-0.15	-0.17	0.42*	-0.06
Clinical mastitis	0.04	0	0.42*	-0.03
MRESMILK ¹	-0.25	-0.18	-0.48*	0.12
Early lactation	0.03	0.15	0.21	0.61*
Soiled udder	0.10	-0.15	0.18	0.61*
Soiled teats	0.16	-0.03	0.17	0.45*
End of lactation	0.12	-0.07	0.06	-0.54*

¹ mean residual of observed-expected milk yield

² mean of logtransformed somatic cell count

Reduced milk yield and high SCC was related to the mastitis udder whereas low SCC was related to small udders. The oblique rotation revealed a low inter-correlation between the components in

general. The highest inter-component-correlation (-.17) was found between component 1 “small udder” and component 4 “soiled udder”. That is, in general small udders were slightly less soiled.

4.4 Udder health parameters in organic farms with non-antibiotic treatment strategy

The organic farmers had on average 0.16 mastitis treatments per cow year; six farms did not have any antibiotic mastitis treatments. Farms keeping DH had more mastitis treatments per cow year than farms with Jerseys or cross breeds (0.23 vs. 0.07, $P < 0.01$). In three farms no cow with a dry quarter was found whereas in other farms up to 18% of cows had a dry quarter. Leg hygiene scoring showed that 18.2 % of the cows had completely clean legs (score 0), 63% had spots with manure at the lower leg (score 1). Only 2 cows had the whole body covered with manure (score 4). 73% of the udders were completely clean, 24% had spots of manure on the udder and 3% had bigger parts of the udder covered with manure. We could not detect a significant correlation or pattern between mastitis treatments per cow year and the prevalence of cows with dry quarters. Three farmers had more than 0.40 mastitis treatments per cow year, two of them with less than 5% cows with dry quarters.

The PCA extracted 2 components that explained 64% of the variation in the data. Component 1 was labelled ‘Soiling, DH, asymmetric quarters & treatments’ because it consisted of the variables herd leg hygiene score and herd udder hygiene score, DH, asymmetric quarters and mastitis treatments. Component 2 was characterized by the prevalence of cows with nodes, prevalence of cows with a dry quarter, and prevalence of cows with knotty tissue, which were interpreted as variables that describe an udder with chronic clinical changes and therefore labelled ‘chronic mastitis udder’.

The relationship between the extracted two components and the CBSCC was examined in a general linear model. Both components showed a significant association with CBSCC that explained 48% of the variation between the farms on CBSCC. An increase of component 1 of 1 unit was associated with an increase of CBSCC by 46,000 cells/ml. Component 2 showed a non-linear relationship with CBSCC. CBSCC increased with component 2 for negative scores, but for scores above 0 no relation was seen. The lowest CBSCC was observed in farms loading high on

component 1 and low on component 2. Farms with negative loadings on both components had medium CBSCC between 200 and 300; only one had a CBSCC less than 200.

The results of the PCA were compared to a model for CBSCC where all variables entered the model individually. Increasing herd leg hygiene score and prevalence of cows with nodes were associated with an increase of CBSCC and explained 44% of the variation between herds on CBSCC. All other variables were not significant in the multivariable model.

5 Discussion

5.1 Study design and data collection

5.1.1 Observational studies

The aim of this thesis was to explore, describe and analyse information from systematic clinical udder examinations, which can be useful as an additional tool in udder health assessment in dairy farms. To achieve this aim, it was important to carry out SCE under real life situations in several study populations with a variety of housing systems and management styles. Therefore, observational studies seemed to be an obvious choice. A further argument for choosing observational studies was the complexity of mastitis and the related risk factors and the interactions between management and udder health. These complex relations could not have been investigated with an experimental study.

5.1.2 Selection criteria of the studied herds

The farms within the different studies were considered to represent a broad spectrum of typical herds in the respective region that are expected to be in operation during at least the next decade. The German farms enrolled in the subprojects 1 (milk leakage) and 2 (primiparous cows) can be considered as typical loose housing systems in the area. All relevant breeds were presented and the farms were located all over Schleswig-Holstein. Farmers that were selected had yearly maintenance of the milking machine, monthly milk recording and a contract with agricultural advisory services. Consequently, the herds represented well-managed herds.

The 16 herds that participated in subproject 3 were selected among herds that were enrolled in a project run by the Danish Dairy Board (Andersen et al., 2000) and located in the geographical ‘Kongedå region’. The study farms had a slightly higher proportion of cows in tie-stalls than the currently 52% reported by Trinderup and Enemark (2003) and mainly kept Danish Holsteins.

The organic farmers in subproject 4 (non-antibiotics-policy) had the common interest to phase out antibiotic treatments in their farms and were all delivering milk to the Thise Dairy. Their attitudes and management style regarding udder health probably cannot be regarded as representative for organic farmers in general, but a wide variety of farming styles were presented regarding breed,

herd size and housing system. These farmers were of special interest to examine the contribution of SCE in udder health assessment, because the commonly used udder health parameter ‘cases of treated clinical mastitis’ obviously had a different interpretation in comparison to herds without the aim to phase out antibiotic treatments. The farmers stated that antibiotic udder treatments were the least favourable treatment option that should be used on emergency cases only. SCE were carried out in these herds with the focus to document udder health and animal welfare under a non-antibiotic treatment policy.

The farmers that showed interest in the studies and accepted the interference of their daily routine and the tasks associated with participation in the studies were included, which may have biased the results. The herds may have been better managed than the general population, e.g. in the German study where good record keeping and collaboration with consultants and veterinarians were essential. As a consequence, cows may have a lower risk of getting mastitis in well-managed herds. But again, the purpose was to study SCE under practical conditions and those farmers who were interested in participating in scientific studies can be regarded as representative for farmers who are open to try new methods in udder health monitoring, in our case SCE. In summary, a wide variety of farms were selected within the different subprojects that represented specific groups of farming styles, which were regarded as representing relevant target groups for the implementation of SCE in udder health monitoring.

5.1.3 Study design and adjustments according to practical circumstances

The schedule of farm visits is often a compromise between research question and practical circumstances. The study design differed in the four manuscripts. SCE were carried out in a random sample of cows as a cross-sectional study in manuscript 3 and 4, with 5 repeated visits in manuscript 3, whereas cows in specific stages of lactation were examined at repeated farm visits in manuscript 1 and 2. With the different sampling strategies, approximately 50% of the cows in manuscript 3 were examined more than once and approximately 30% of the cows in manuscript 1 were examined in two subsequent lactations. Therefore, the variation within cows could not be analysed effectively and only one observation per cow was chosen for statistical analysis.

In contrast to other studies that used the farmers’ observations of the cows in the postpartum period (Slettbakk et al., 1995; Waage et al., 2001) we could not detect a relationship between clinical

mastitis, milk leakage and peripartum oedema. The study design did not allow to examine the cows at specific days of lactation, e.g. at calving. SCE were exclusively carried out by the author or trained technicians during farm visits, but farm visits at each calving were not feasible with the available resources for our studies. If the association between milk leakage, udder oedema and mastitis should be the focus of research, the farmers should be trained to examine and record the clinical variables. The major disadvantage of this approach is that it is virtually impossible to differentiate between observer and management effect. The different thresholds or ability to assess the clinical features would have influenced the results. Our results would have been dependent on the farmer's compliance and could result in excluding farms that did not examine and record regularly. Farmers may even retreat from a project where they have to carry out clinical examinations at specific stages of lactation because of the workload. Farms with incomplete data recording are often the most challenging ones with regard to presence of disease problems and epidemiological findings, but have to be excluded. Barkema et al. (1998) excluded 18 of 304 herds because of insufficient compliance. In the three examined study populations no farm was excluded and no farmer retreated from the collaboration.

5.1.4 Clinical variables and observer effect

The clinical variables used in this thesis were from the three focus areas: morphology of udder and teats, milking related or environmental changes, and pathological changes caused by infections. In common for all studies was that the examination was based on inspection and palpation without using other instruments than eyes and hands. Inspection and palpation of patients are basic skills for clinicians in diagnosing diseases in individual animals. With SCE these skills are extended from the individual cow to the herd as unit of interest. Visual assessments of cows are widely used, e.g. linear type traits in breeding programs, where clinical observations often are regarded as subjective because of the observer effect (Veerkamp et al., 2002). Kristensen et al. (submitted) found excellent agreement between experienced classifiers in body condition scores (kappa values around 0.9). Furthermore, the authors report a substantial improvement in validity and precision after a limited training of inexperienced veterinarians. In order to improve within- and between-observer agreement (manuscript 3), common training sessions, discussion and adjustment of the recording sheet after the first round of farm visits had taken place. The last observation per cow was regarded as the most valid and precise and used for the analysis.

The advantage of examination by inspection and palpation is that the examination can be done easily, within a short time during the milking, independent of other measuring instruments, and with acceptable interference of the milking staff. Practicability and feasibility are of great importance with regard to acceptance and implementation of SCE in practice. We would maybe have had some ‘more significant’ results with continuous measurements e.g. regarding teat length and diameter and udder-floor distance. To minimize within and between observer bias training, well-described variable levels and schemes are essential. Another potentially very important bias is the so-called ‘frog pond effect’ that is widely recognized in social sciences. This term describes the phenomenon that a frog living in a pond with small relatives is perceived as being larger than the same frog living in a pond with large relatives, i.e. the perception of the size of the frog can depend on the frog’s social context (Kreft et al., 1995). In terms of clinical observations, observers may assess clinical conditions in cows dependent on the conditions of the other cows within a herd. The presence of this bias may be detected by including herd level means of the variable of interest as a predictor in the model (Kreft et al., 1995).

5.1.5 Automatic milking systems as example for adjustment of SCE according to practical circumstances

Depending on the number of clinical variables and the sample size of cows, the SCE prolonged milking duration in the milking parlour up to half an hour. Two of the farms in manuscript 4 operated with an AMS. In farms with robotic system the udders of the cows show different degrees of udder filling because of individual milking intervals. The cows develop individual feeding patterns (Melin and Wiktorsson, 2004). Especially in forced cow traffic where cows only get access to the feeding area after passing the automatic milking unit (AMU), the cows’ possibilities to synchronize their behaviour is limited (Hermans et al., 2003). The SCE has to be done still allowing the majority of cows to keep their daily pattern of feeding, drinking, milking and lying. To restrain the cows at the feeding gate for the SCE for up to two hours would have disturbed the cow traffic and exploitation of the robot considerably. In the farm with forced cow traffic and cubicle housing, the SCE was done in the cubicles, and individual cows were gently driven to the cubicles if necessary. With this practice 50 cows were examined in 3 hours, i.e. the duration of the SCE was considerably longer than in milking parlours, but interference of the cow traffic was low. One farm

operated the AMU in a deep litter system where SCE had to be carried out in the AMU. A maximum of 10 cows/hour can be examined. The sample size and criteria of sampling have to be assessed carefully because of the high personnel costs. To keep SCE feasible in robotic systems, the sample sizes should be reduced selecting groups of cows at the highest risk of mastitis, e.g. cows in early lactation. The cows can be selected automatically to the separation units after milking, where the SCE is carried out.

5.2 Challenges in statistical modelling

5.2.1 Quarter level

In the statistical analysis in manuscript 1 and 2, the correlation between the quarters within a cow (“clustering”) had to be considered. In manuscript 1, the binary outcome milk leakage occurred most often in all four quarters of a cow. Many of the potentially important risk factors for milk leakage, i.e. udder and teat shapes and teat condition were measured on quarter level. In most cases classification of all quarters within a cow was similar, in some cases the quarters were scored differently. If differences were observed, it was mainly between hind and frontquarters. In the initial risk factor analysis where all quarters within cow were included, under-dispersion was observed. Under-dispersion indicates less variation in the data than expected by chance (Brown and Prescott, 1999). When risk factors for milk leakage were analysed, the problem of under-dispersion was solved using one randomly selected quarter of a cow. This method can be an alternative if the examined diseases/outcome of interest mainly affects all quarters. A modified method was used in the risk factor analysis for IMI and clinical mastitis (manuscript 2). In this study the outcome variable occurred on quarter level, but similar to manuscript 1, most cows showed the same scores in the clinical variables (the risk factors). In this case, the problem of under-dispersion was solved by selecting an infected quarter in the infected cows and one random quarter of the healthy cows. The disadvantage of this approach was that the effect of spreading of the infection between the quarters of a cow could not be assessed.

5.2.2 Definition of cases of clinical mastitis

To demonstrate the association between teat characteristics and mastitis in manuscript 2, clinical cases of mastitis were defined as those that were treated by the farmer or the veterinarian. The

farmers' records were checked and the farmers interviewed monthly to achieve a high data quality. All farmers stated that they treated all observed clinical cases of mastitis in early lactation of first lactation cows. Consistent with findings of Vaarst et al. (2002), the farmers had a high willingness to treat the observed clinical cases of mastitis in first lactation cows. The records of veterinary treated cases of clinical mastitis in manuscript 4 were extracted from the Danish Cattle Database. Under-reporting of treated cases of clinical mastitis to the Danish Cattle Database has been shown (Bartlett et al., 2001). Bennedsgaard (2003) checked farm records, withdrawal notes and the veterinarians' diaries and compared the written sources with the registrations in the Danish Cattle Database. He reported an underestimation of treatment frequency of about 20% when the uncorrected data were used. The group of farmers participating in the project to phase out antibiotic treatments were highly motivated. Some of them started the process to promote health and reduce veterinary treatments several years before the project period. We do not think that under-reporting was present in the studied herds because the farmers actively used the frequency of antibiotic treatments to monitor the success of their health promotion and to critically assess in which stage of the process of phasing out antibiotics they were in (Vaarst et al., 2005).

Milk leakage

Even though milk leakage was not related to the risk of getting mastitis in manuscript 2, the hygienic problem remains. Probably the risk for other cows in the herd than the affected animals is as relevant as or even more relevant as the risk for the affected cow. The risk could be dependent on cubicle design and management on the one hand and the cows' lying behaviour on the other hand. Milk is rich of nutrients for bacterial growth. Herd mates lying in contaminated milk probably are at higher risk for mastitis. Furthermore, milk-leaking cows increase the bacterial burden in the barn, which may lead to an increased risk for mastitis in all cows.

Further research is necessary to enlighten these relationships. In cows with milk leakage the amount of milk obtained by the farmer decreases. On the other hand in cows with milk leakage, the IMP decreases which may enhance milk production (negative feedback of IMP on milk production).

Leakage was measured when cows entered the milking parlour. Peeler et al. (2000) questioned the farmer if they had cows in the herd that leaked milk when entering the milking parlour. The presence of cows leaking milk was associated with a higher risk of clinical mastitis (farmers' reports) in herds with low bulk somatic cell count (Peeler et al., 2000). The authors reported a higher risk when cows were leaking milk at other times of the day. The analysis was carried out on

herd level, and the relationship between leakage and clinical mastitis may be different when analysed on the individual cow level (Dohoo et al., 2003).

Peak milk flow

Peak milk flow was a strong predictor for milk leakage and IMI with *Strep. uberis* (manuscript 1 and 2). Peak milk flow is influenced by milk yield (Johansson and Malven, 1960), the applied vacuum and the size of the teat orifice (Johansson and Malven, 1960). Peak milk flow defined as the maximum milk harvested during a 1-min interval has a high repeatability within parity (0.95 within parity 1) and between parities (0.95-1) (Petersen et al., 1986). Screening for cows deviating from the herd mean peak milk flow adjusted for milk yield and stage of lactation will provide important information on cow specific risk factors (teat canal patency), dimensions of the milking machine (transport of harvested milk, back flush of milk – cross-contamination, residues after milking as risk for subsequently milked cows etc. In AMS peak milk flow is measured at each milking and can be used to assess teat canal patency and hereby the risk for milk leakage and IMI with *Strep. uberis*. In farms that do milk yield control with the Lactocorder, the information on peak milk flow and other milking characteristics can be retrieved by the control organization. As demonstrated in manuscript 1 and 2, will the assessment of the adjusted peak milk flow in first lactation cows and incorporation in on-farm breeding plans most likely be beneficial to control milk leakage and the risk for IMI with *Strep. uberis*.

Parity effects

Lactation curves, udder size and teat characteristics differed between parities (manuscript 1 and 3). To solve problems caused by multicollinearity and confounding as observed in manuscript 1, primiparous and multiparous cows were analysed separately. Manuscript 1 and 3 stress the importance of considering age related differences of various manifestations in research as well as in udder health assessment on farm.

5.3 Udder health indicators for udder health assessment

5.3.1 Chronic udder type

The chronic udder type was extracted by PCA in manuscript 3 (cow level, related to SCC) and manuscript 4 (herd level, related to CBSCC). The chronic udder type may be important for udder health assessment, especially in farms that operate with a non-antibiotic treatment policy. The prevalence of cows with dry quarters and the treated cases of mastitis/cow year gives information on which type of treatment is used to which extent. In combination with the prevalence of cows with nodes in the udder we could assess the success of the treatment policy with regard to CBSCC. A high prevalence of cows with dry quarters in combination with low CBSCC would indicate that the prevalence of mastitis is high, but the treatment in terms of control of CBSCC is effective.

The prevalence of cows with a dry quarter could relatively easy be obtained in farms with good record keeping and in farms operating with AMS. Asking the farmers may be insufficient when no records are kept and cows simply marked on their legs. Webster et al. (2004) found a good association between the farmers' estimates of mastitis incidence and records of treatment. They concluded that the farmers had a policy to treat mastitis early after detection and record each treatment. A further motivation for good record keeping may be the economic consequences if the milk of treated cows accidentally gets in the bulk tank. The situation may be different for cows with a dry quarter, where an accidental milking of the dry or to be dried off quarter will not have other consequences than an eventually higher BMSCC. SCE during milking would be necessary in these farms.

5.3.2 Principal component analysis to identify patterns

PCA was carried out to detect cow patterns and herd specific patterns. In manuscript 3 PCA extracted 4 udder types from 23 variables describing different udder and teat characteristics and the relation between them and production parameters (milk yield, SCC). In manuscript 4 PCA extracted two different types of herd characteristics. PCA was a useful tool to reduce the number of variables, to reveal the relation between the relevant variables, and to recognize patterns. PCA can be a useful tool for on-farm analysis of SCE in order to identify herd specific patterns.

5.3.3 Hygiene score

Herd leg hygiene scores and udder hygiene scores were related (component 1 in manuscript 4), but only the herd leg hygiene score was identified as important risk factor for high CBSCC (manuscript 4). Therefore, the leg hygiene score should preferably be used as indicator for udder health and to monitor the effect of changes in management on the cleanness of the cows. On cow-level, soiled udders are related to early lactation (manuscript 3). On herd-level (manuscript 4), a lower mean DIM at the time of the SCE was associated with higher udder and leg hygiene scores (= higher degree of soiling with manure). When hygiene scores are applied in udder health management, the scores should be adjusted for stage of lactation.

The aim in manuscript 4 was to detect udder health indicators on herd level in order to identify the applied management strategies, to classify the udder health on herd level and to develop methods to be used in udder health management on herd level. Because all cows within a farm were housed, fed and milked under similar conditions, we assumed little variation in the exposure to mastitis. Therefore, a herd-level analysis was regarded as a relevant method, even though the number of herds was limited and results have to be interpreted with caution. As mentioned above with the case of milk leakage (page 56), ecologic bias may occur. This is the assumption that an observed relationship in aggregated data (herd level) may hold at the individual level (Dohoo et al. 2003). The clinical variables were analysed on the cow level (manuscript 3) and on the herd level (manuscript 4) in two different study populations. As demonstrated above with the chronic udder type and hygiene scores, are the findings in both approaches similar.

5.3.4 Teat characteristics

Teat characteristics were related to milk leakage, IMI, and CM. Typically cattle breeding organizations, in Denmark the Danish Cattle Federation, are responsible for the classification of udder and teat conformation to construct breeding indexes. Only a selected number of herds have a herd classification on a regular basis. In herds with systematic classification of first lactation cows the information on udder and teat conformation could be used to assess pathogen specific risks of mastitis in a herd. In farms not classifying cows systematically, udder size, teat shape, teat and teat end shape should be included in the clinical protocol for SCE.

5.4 Implementation of systematic clinical examinations in udder health monitoring

Are systematic clinical udder examinations already applied in practice?

Animal based observations to assess health and welfare become more popular in dairy farming systems in several countries. Clinical protocols have been developed to assess animal welfare or production quality (Webster et al., 2004; Noordhuizen and Metz, 2005). Some clinical protocols also include udder health indicators. In Sweden, 'FRISKKO-JUVER (HealthyCow - Udder), an udder health prevention program has been launched where herd data and routines are evaluated in 4 steps. The animal based observations systematically used in the program are: body condition score, cleanness and teat condition (Ekman et al., 2005). Common for these systems is the aim to document to consumers and authorities a certain standard of animal welfare. Teat club international promotes systematic teat health assessment as tool to improve udder health and supplies recording sheets and spread sheets that can be used to analyse and determine herd specific teat health problems (Anon., 2005a).

Danish veterinarians who are specialised in herd health management have offered systematic clinical examinations as a part of the health-and production analysis in dairy herds since 1997 (Anon., 2005b). The concept is inspired by the services offered by the cooperative Israelian veterinary service (Nir, 2004). All cows in specific risk groups are systematically examined at (weekly) farm visits. The clinical findings are recorded and form a major part of a comprehensive health and production analysis. Udder health is assessed by inspection and palpation of the gland and CMT.

A few studies deal with the udder as a whole, combining conformation, pathologic and environmental changes to work on the udder health concept (what is normal). Clinical protocols with focus on udder health assessment that include indicators concerning udder and teat conformation, environmental or milking induced changes and pathologic changes in udder and teat have been applied by Houe et al. (2002) and Bruun (2003).

The effect of combining phenotypical and genotypical clinical features in SCE to improve udder health management should be further explored. Conflicting results within the literature regarding the relation between udder and teat conformation and SCC could be explained by the different pathogens in different populations, modified by the breed, system and the applied management. Some of the differences in treatment patterns and mastitis incidences within the Nordic countries as

reported by Valde et al. (2004) could be due to different genetic characteristics of the studied populations and breeds, and the interaction between genetic characteristics and the different distribution of mastitis pathogens between countries.

Practical implementation of clinical examinations in health advisory service

Because of the multifactorial nature of mastitis, a mastitis control program is complex as well and should cover the whole farming system. Therefore, SCE should be implemented in herd health programs and results from clinical udder examinations seen in context with milk recordings and farm documentation.

SCE could be valuable in several steps of developing a herd specific mastitis control program: definition of the udder health problem, risk factor identification and the relevance of the risk factors within the herd context. SCE can help to identify groups at risk for different forms of mastitis. The number of cows with chronic udder type (manuscript 3 and 4) reveals whether blinding of a quarter is practiced as mastitis treatment. In combination with the veterinary treatment records the importance of CM in the herd can be estimated. Combining this information with the CSCC would give an estimate about which type of mastitis is predominant and how effectively mastitis is managed. The evaluation of phenotypic features in relation to the predominant mastitis pathogens could be used for breeding recommendations (long-term) and to focus on the effective farm specific prevention methods (short-term). The proportion of cows with teat damage due to deficiencies of the milking could effectively be used to monitor milking technique and milking practices. Furthermore, clinical parameters like milk leakage, teat end condition, and teat morphology give the direction for the focus of a farm specific udder health plan. According to a farm specific problem and risk factor profile, relevant clinical parameters can be chosen for further monitoring.

SCE can be a useful tool to compare udder health conditions between farms, given that the levels of the specified clinical variables are standardized, that is, benchmarking. In this context, benchmarking can demonstrate what can be achieved and motivate farmers to improve udder health. Huxley et al. (2004) investigated animal welfare assessment benchmarking as a tool for farmers for the development of animal health and welfare plans. The concept and examples for problem identification and solutions can be downloaded from the internet (Main et al., 2004).

SCE can be carried out by trained technicians, veterinarians, and other advisors as well as by the farmer. The farmer could easily obtain most of the clinical parameters used in this thesis during milking; often he or she observes them anyway. The challenge is that farmers should record the relevant parameters in a systematic approach, so that they become information that can be used as management tool. A Dutch survey (Kuiper et al., 2005) showed that nearly all farmers monitor BMSCC, individual SCC and the development of new cases of clinical mastitis. Though all farmers regarded mastitis management as an important topic, only 25% of the farmers had an actual mastitis management plan and only 6% had the plan written down. One way of promoting the implementation of systematic udder health plans in farms will be a better coordination of udder health management activities, which implies collaboration between the farmer and all enrolled experts and a structured approach (Nordhuizen, 2005). While the Dutch farmers stated that the veterinarian was the most important source of information to increase their knowledge about mastitis (Kuiper et al., 2005), reported Vaarst et al. (2003) that the veterinarians in organic farms after conversion were mainly restricted to the treatment of individual cases of clinical mastitis and rarely involved in herd level perspectives.

The veterinarian can and should play a central role in developing udder health plans that include SCE. The trend towards larger herds and higher production most likely will continue in the future demanding a systematic approach for the veterinarian. Not only retailers or non-government organizations, but also authorities are getting more interested in the potentials of SCE in monitoring health and welfare. A project has been carried out in Denmark to evaluate the quality and feasibility of clinical recordings made by veterinarians together with other data. SCE of pre-defined high-risk groups were an essential part of the veterinary herd health monitoring. The results will be a basis for a modified legislation regarding herd health contracts between veterinarian and farmer (Anon., 2005c). The future veterinarian should be highly skilled not only in diagnosing and treating diseases on cow level, but also on herd level. A profound knowledge about cow and herd data analysis is essential. SCE as an integrated part of an udder health monitoring system will be a valuable tool for veterinarians offering advisory service in modern dairy farming. With systematic collection of udder and teat characteristics the veterinarian can compare udder health in the different herds, motivate farmers and assess the effect of his/her advice. In order to promote the use of SCE in practice, SCE should be implemented in the education of veterinarians.

6 Conclusions and future research

6.1 Conclusions

Main conclusions from the studies presented are:

Udder health indicators

- Teat canal protrusions and high peak milk flow increase the risk of milk leakage in primiparous and multiparous cows. To reduce the prevalence of cows leaking milk, breeding programs should consider risk factors for milk leakage. Additionally, screening of multiparous cows for short teats and inverted teat ends may be beneficial to evaluate the risk for milk leakage. Because most of the variation in the data occurred on the cow level, assessment and selection of individual cows is necessary in order to reduce the occurrence of milk leakage in the herd.
- Important risk factors for *Strep. uberis*-infection were deviance from the average milk flow, calving in winter and condition of the teat orifice. The risk of IMI with *Staph. aureus* was higher in cows with short and thick teats, and in cows with pointed teats. Significant risk factors for clinical mastitis were calving in winter, hindquarters, deep udders and large teats. The different udder and teat characteristics should be considered in breeding plans.
- The chronic udder type consisted of clinical variables that described pathologic changes in the udder and was related to somatic cell count on cow and herd level.
- Leg hygiene and udder hygiene scores were related to each other and to early lactation. Leg hygiene scores were also related to calculated bulk somatic cell count. Leg hygiene scoring will be an effective tool in udder health monitoring. The results should be adjusted for stage of lactation.
- Under a non-antibiotic treatment policy, the method of drying off infected quarters was used as a management tool comparable to using antibiotic treatments. Therefore the prevalence of dry quarters has to be interpreted in relation to other udder health indicators to assess udder health in a specific farm.

Statistical modelling of udder health

- Principal component analysis is an effective method to identify patterns within the clinical variables and in combination with production data, which provides important information on udder health in addition to treatment data or data from the milk recording. The complete extracted components can be used for pattern recognition as diagnostic reasoning strategy on farm. Secondly, PCA can be used to select the most important clinical variables in order to simplify the clinical examination protocol.
- In non-contagious outcomes such as milk leakage, a condition most frequently observed in all four udder quarters, the random selection of one quarter as in paper 1 can be a solution to allow utilization of the information on quarter level.
- Primiparous and multiparous cows are different with regard to lactation curve, teat shapes and udder size, which has to be considered in statistical analysis. In conclusion, primiparous and multiparous cows should be analyzed separately and caution paid when analyzed together.

Application of systematic clinical udder examinations

- Systematic clinical examinations of udders contribute to the understanding of udder health, reveal farm specific mastitis treatment patterns and farm specific risk factors. Therefore it will be beneficial to use SCE as additional tool in udder health management. To apply systematic clinical examinations of udders in dairy herds a detailed protocol is necessary as well as an intensive training of the clinicians examining the udders. The point of time when to examine the udders, the number of consecutive examinations per cow and the number of cows selected in the herd has to be considered carefully.
- SCE can be used for benchmarking

6.2 Future research

- Methods for an efficient and feasible analysis of SCE data on farm, e.g. for the veterinarian should be further explored.
- The economic benefit of including SCE in udder health plans should be studied.
- The effect of implementation of SCE in different farming systems should be further studied with regard to acceptance of the method, optimal choice of herd-specific indicators and follow-up.
- The relationship between udder and teat morphology and pathogen specific mastitis should be explored in a larger study to assess the effect of implementing the relation in breeding programs.

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Due to restrictions from the publisher of the journal in which manuscript 1 and 3 have been published, these articles are not available in this PDF. The articles can be found in:

Manuscript 1:

Klaas, I. C., Enevoldsen, C., Vaarst, M., & Houe, H. (2004). Systematic Clinical Examinations for Identification of Latent Udder Health Types in Danish Dairy Herds. *Journal of Dairy Science*, 87, 1217-1228.

Abstract:

Milk leakage in dairy cows is a symptom of impaired teat sphincter function. Milk leakage is related to an increased risk of mastitis in heifers and cows, and causes hygiene problems. The aim of our study was to assess whether teat shape, condition of teat orifice, and peak milk flow rate are risk factors for milk leakage. We conducted a longitudinal observational study in 15 German dairy farms in which cows were maintained in loose housing. The farms were visited monthly at 2 consecutive milkings. During the evening milking, milk flow curves were measured with the LactoCorder. Milk leakage was recorded during the subsequent morning milking, when cows entered the milking parlor. Immediately after detachment of the milking cluster, teat shape, teat end shape, and condition of the teat orifice of cows were assessed between 9 and 100 d in milk (DIM) and during late lactation (>250 DIM). Data from 1600 cows were analyzed. Milk leakage was treated as the binary response variable in a logistic regression model with herd as a random effect. Primiparous cows with high peak milk flow and teat canal protrusion were at greater risk of milk leakage. High peak milk flow rate, short teats, teat canal protrusion, inverted teat ends, and early lactation increased the risk of milk leakage in multiparous cows. Random herd effects accounted for only 10% of the total variation, indicating that the impact of management or other herd-level factors on the occurrence of milk leakage is virtually negligible for practical purposes.

Abbreviation key: **DevMilk** = deviation from the population mean milk yield adjusted for stage of lactation and parity, **DevPMF** = deviation from the population mean peak milk flow adjusted for stage of lactation and parity, **MeanResMilk** = mean residual of observed –expected milk yield

Manuscript 3:

Klaas, I. C., Enevoldsen, C., Ersboll, A. K., & Tolle, U. (2005). Cow-Related Risk Factors for Milk Leakage. *Journal of Dairy Science*, 88, 128-136.

Abstract:

A cross-sectional study was conducted to explore the applicability of systematic clinical examinations of udders as an additional tool for the evaluation of udder health status on dairy farms. During 2000, each of the 16 dairy farms was visited 5 times; 20 cows per farm were chosen at random at each visit for clinical udder examination immediately after milking. The clinical examination included both pathological and morphological variables. One examination per cow was included in the analysis (n = 707 cows). Principal component analysis (PCA) was performed in 3 steps. First, 19 variables characterizing udder and teats were analyzed (PCA 1). Second, the variables parity and stage of lactation were included (PCA 2). Finally, somatic cell count (SCC) and milk yield (PCA 3) were included. The PCA resulted in 4 components that explained 30% of the variation of the data: 1) small udder, 2) distressed udder, 3) mastitis udder, and 4) soiled udder. Variables with high positive correlation to the "small udder" were small udder shape, short teats, and first parity. Impaired teat surface, hard udder texture, and a long udder shape were related to the "distressed udder." The "mastitis udder" was characterized by the clinical variables asymmetry between front quarters, asymmetry between hind quarters, knotty tissue, and acute clinical mastitis. Reduced milk yield and high SCC were related to the "mastitis udder," whereas low SCC was related to the "small udder." The "soiled udder" was related to early lactation. Including this information in the assessment of udder health may be of substantial value for data analysis in farms with suspected under-reporting of clinical mastitis.

Abbreviation key: **ECM** = energy-corrected milk, **MLOGSCC** = mean of log-transformed SCC, **MRESMILK** = mean residual of observed-expected milk yield, **PCA** = principal component analysis

RISK FACTORS IMI PRIMIPAROUS COWS

Cow-related Risk Factors for Intramammary Infections with *Streptococcus uberis* and *Staphylococcus aureus* in Primiparous Cows

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ABSTRACT

An observational study was conducted to identify udder and teat characteristics that are related to a higher risk of intramammary infections (IMI) and clinical mastitis in primiparous cows. Milk samples were obtained from all lactating cows in 15 commercial dairy farms at 4 weeks intervals over a period of 15 months. Udders and teats of all primiparous cows in early lactation were clinically examined immediately after milking. Milk flow curves of all cows were recorded and data concerning diseases and treatments of cows were collected.

The predominant major mastitis pathogens were *Strep. uberis*, and *Staph. aureus*. Logistic regression with herd as random effect was carried out to identify risk factors for clinical mastitis and IMI with *Staph. aureus* and *Strep. uberis* during the first 100 days of lactation. Important risk factors for *Strep. uberis*-infection were deviation from the average milk flow, calving in winter and condition of the teat orifice. The risk of IMI with *Staph. aureus* was higher in cows

with short and thick teats, and in cows with pointed teats. Significant risk factors for clinical mastitis were calving in winter, hindquarters, deep udders and large teats. The proportions of variance due to herd were low. Herd prevalence of infected quarters in the preceding month reduced the random herd effect in the models for *Strep. uberis* and *Staph. aureus* considerably. The herd effect contributed to 17% of the variance in the model of clinical mastitis. In conclusion, different teat and udder characteristics are risk factors for different types of mastitis. Recordings of udder and teat characteristics seem to be important for effective management of pathogen-specific mastitis risks in the herd and for breeding purposes.

(Keywords: udder conformation, teat conformation, *Staphylococcus aureus*, *Streptococcus uberis*, primiparous cow)

Abbreviation key: DevPMF = Deviation from average peak milk flow adjusted for milk yield, DIM = days in milk, IMI = intramammary infection, PMF = Peakmilkflow

INTRODUCTION

Even though considerable efforts have been made to control mastitis under field conditions, mastitis remains to be the most important production disease in dairy cows (Seegers et al. 2003). The aetiology of mastitis is complex, as it involves the cow, the pathogen and the environment (Tolle et al., 1977). The cow's resistance against mastitis is determined by genetic and physiological components and influenced by environmental factors (Rupp and Boichard, 2003). Because the main route of infection is the teat canal, the conformation of udder and teats may play an important role in the pathogenesis of mastitis as they can facilitate bacterial growth and the physical penetration of the teat canal (Tolle et al., 1977). Many studies, experimental and observational, have examined the association between udder and teat conformation and clinical mastitis and SCC, sometimes with conflicting results (Seykora and McDaniel, 1985). Possible explanations for these discrepancies may be differences in variable definition, study design, sample sizes and management practices, as well as different mastitis pathogens in the different study populations. Bakken (1981) found a higher incidence of *Staph. aureus* mastitis in cows with funnel

shaped teats. Recently, rough teat end callosity has been reported to increase the risk for *Staph. aureus* mastitis (Zadoks et al 2003).

Strep. uberis is a common environmental pathogen with organic bedding as a common source of infection (Bramley 1982, Hogan et al. 1989). Breeding for milking ease, a wider and shorter streak canal has increased the infection risk for *Strep. uberis*. In an experimental study, cows with higher peak milk flow experienced a higher incidence of *Strep. uberis* infection whereas a longer teat canal was protective against *Strep.-uberis*-infection (Lacy-Hulbert and Hillerton, 1995). There is some evidence that the specific pathogens are related to different morphologic features. If specific udder and teat characteristics are related to a higher risk of pathogen specific IMI and clinical mastitis, a systematic assessment and subsequent recording of udder and teat characteristics can be used to develop pathogen-specific management regimes. Furthermore, we may be able to select more resistant cows and manage high-risk cows selectively.

The objectives of our field study were to estimate the associations between typical udder and teat characteristics and the risk of IMI with *Strep. uberis*, *Staph. aureus* and the risk of clinical mastitis during the first hundred days of lactation in first lactation cows.

MATERIAL AND METHODS

Our observational study was carried out in 15 commercial dairy farms in Schleswig-Holstein, Germany. The herds were enrolled in a project to improve udder health. Criteria for inclusion in the study were that the farmers participated in a milk-recording scheme, were advised by agricultural advisory services, conducted regular maintenance of the milking machine and were willing to record all veterinary and owner treatments of diseases during the study period. Thirteen farms operated with a herringbone-milking parlor, and two farms with a tandem parlor. Table 1 shows the herd characteristics. All herds were housed in free-stall barns with cubicles and slatted floors, and were grazing during the summer period (May-September). The first author visited the farms monthly from September 1997 until December 1998. Milk samples were taken aseptically of all lactating udder quarters for bacteriological examination, and milk flow was measured. The udders and teats of first lactation cows in early lactation were examined and data on disease occurrence and treatments were collected.

Table 1. Characteristics of 15 dairy herds.

Herd	Cow- years	Breed ¹	ECM ² (305 d)	SCC ³	Healthy cows	Cows with Strep. uberis	Cows with Staph. aureus	Cows with CM ⁴
1	63	RH	7710	145	30	1	2	2
2	64	HF (cross)	9809	150	6	1	1	5
3	70	HF	8882	139	11	0	3	4
4	56	Angler	8576	187	10	2	0	4
5	70	HF	8165	179	11	2	0	5
6	100	Angler	8566	143	31	1	4	4
7	66	HF (cross)	5287	187	6	0	2	7
8	110	RH (HF)	5671	247	34	3	9	5
9	99	RH (cross)	6906	344	19	2	1	2
10	46	RH, (HF)	7895	81	7	2	0	1
11	85	HF, RH	8895	172	12	4	0	4
12	53	RH	6245	253	8	0	1	3
13	87	HF (cross)	6778	140	19	1	0	0
14	70	HF	9124	160	10	1	0	2
15	92	HF, Angler (cross)	7793	148	16	6	5	4

¹ Dominant breed in herd, minor breeds in brackets; HF = German Holstein Friesian, RH = German Red Holstein.

² Energy corrected milk yield.

³ Calculated bulk tank somatic cell count in 1000/ml.

⁴ Clinical mastitis.

Peak milk flow

During the study period milk recording was carried out with the LactoCorder (Werkzeug- und Maschinenbau Berneck AG, Baldach, Switzerland). In addition to milk yield, the LactoCorder measures the milk flow throughout the entire milking. Milk flow characteristics are measured every 0.7 seconds and saved with value changes or in intervals of 2.8 seconds. Peak milk flow is defined as the maximum milk flow within 8 measurements (= 22 seconds) and given in kg/min. The maximum milk flow is usually measured during the plateau phase of the milk flow curve.

Milk samples

Every month the first author and trained students collected quarter foremilk samples aseptically during the morning milking subsequent to the milk recording by the Lactocorder. After udder preparation by the farmer and after discarding of the first two streams of milk, approximately 30 ml of milk were sampled in tubes containing boric acid for preservation. Except on Saturdays, when samples were stored in a refrigerator until processing on the following Monday, the samples were transported to the diagnostic laboratory within 3 hours after sampling and cultured immediately. Samples were inoculated by streaking onto blood and MacConkey agar plates and incubated at 37°C for 48 h. Mastitis pathogens were identified according to the standards of the German Veterinary Society (DVG-Leitlinien 2000).

Clinical udder examination

The first author examined the cows clinically immediately after detachment of the milking cluster. The udders and teats of all first lactation cows < 100 DIM were examined. The classification of teat shape and teat end shape was based on Rosenberger (1979). Teat orifice was classified as normal, teat canal protrusion, white smooth callosity ring, or rough callosity ring. The definitions of teat characteristics are summarized in Table 2.

Table 2. Definition of teat characteristics and udder size for all cows with or without milk leakage included in the analysis (based on Rosenberger 1979).

Variable	Levels	Definition
Teat shape	Short and thin	Length < 4.5 cm and diameter < 2 cm
	Short and thick	Length < 4.5 cm and diameter ≥ 2 cm
	Normal	Length 4.5-6.5 cm, diameter 2-3 cm
	Conical	Diameter teat end < diameter of teat base
	Long & Thick	Length > 6.5 cm, diameter > 3 cm
Teat end shape	Inverted	Slightly or clearly inverted
	Flat	Flat or slightly plate like
	Round	Rounded or slightly pointed
	Pointed	Clearly pointed
Teat orifice	Normal	Intact orifice, no lesions
	Protrusion	Protrusion of teat canal like a thin pink ring, on black teats thin grayish ring
	White callosity ring	White thick ring with smooth surface
	Rough callosity ring	White thick ring with cracked surface

Teat characteristics were recorded on quarter level. Udder size was measured on cow level and in relation to the hock (Table 2).

Criteria for inclusion in the analysis

Records from cows within the first 100 DIM were included. A record of a cow that left the herd before 100 DIM was included if the cow had clinical mastitis, IMI with *Strep. uberis* or *Staph. aureus*. The cows were divided in four mutually exclusive groups according to the following udder health states:

Healthy cows

Healthy cows were observed for at least 100 DIM in the herd during the study period. The cows were free of clinical mastitis and IMI with major pathogens. They may have had infections with minor pathogens.

***Strep. uberis*-cows**

The first isolated major pathogen in any quarter of a cow was *Strep. uberis* and no clinical signs of mastitis were present. Cows may have had an infection with another major pathogen or may have shown clinical signs of mastitis later in the lactation.

***Staph. aureus*-cows**

The first isolated major pathogen in any quarter of a cow was *Staph. aureus* and no clinical signs of mastitis were present. Cows may have had an infection with another major pathogen or may have shown clinical signs of mastitis later in lactation.

Clinical mastitis cows

Clinical mastitis was recorded by the farmer if the cow showed abnormal secretion and /or udder changes (swelling, warmth, redness, pain). Only the first case of clinical mastitis was included in the analysis. All first cases of clinical mastitis were treated with local and/or systemic antibiotics. Cows in this group may have been infected with major pathogens after the episode of clinical mastitis had occurred.

Exclusion criteria:

Cows infected with other major pathogens than *Staph. aureus* or *Strep. uberis* were excluded from the logistic regression because the incidence of IMI with other major pathogens was too low to allow a meaningful analysis.

Definition of the risk factors

Deviation from the mean peak milk flow (DevPMF)

Peak milk flow (PMF) was taken from a measurement within the study period, when the cow was free of mastitis pathogens and clinical symptoms of mastitis. To adjust for the correlation between peak milk flow and milk yield (Pearson correlation coefficient 0.71, $P > 0.001$), the average PMF within four milk yield classes was calculated (< 6 , 6-10, 10-13, and > 13 kg milk). DevPMF was the deviation from the mean peak milk flow within milk yield class in kg per minute.

Herd prevalence

Sterile quarter milk samples from all lactating cows were examined bacteriologically every month. The herd prevalence was the prevalence of quarters infected with the respective pathogen on a specific milk test day within herd.

Herd prevalence of infected quarters = $\frac{\text{number of quarters infected with pathogen}}{\text{number of sampled quarters}} \times 100$

For cows with an IMI with *Strep. uberis* or *Staph. aureus*, the herd prevalence in the month preceding the infections was used as a risk factor in the analysis. For uninfected cows the herd prevalence was the prevalence of the month preceding the last milk sampling in the observation period.

Calving season

Calving during the winter period (October-April) vs. calving during the summer period where cows were on pasture (May – September).

Data analysis

Complete data of 363 primiparous cows was analyzed using logistic regression with the binary response variables IMI with *Strep. uberis*, IMI with *Staph. aureus* and clinical mastitis. Herd was specified as random effect and estimation was carried out using the Glimmix-macro in SAS (Littell et al., 1996).

In initial analyses we included data from all quarters and specified cow as a random effect. This resulted in serious under-dispersion because there was very little variation between the quarters of a cow. However, within cows, there were some differences in teat shape, teat end shape and teat orifice level. Because the levels in these categorical variables could not be regarded as linear, an aggregation on cow level as a cow-median or cow-mean was not interpretable. Therefore, we decided to select one quarter per cow and to use one observation per cow because this approach permitted us to evaluate quarter level risk factors. In cows where one quarter was affected with IMI or CM, the affected quarter was chosen for analysis in the model. In cows where more than one quarter was affected with IMI or CM, one of the affected quarters/cow was chosen at random. In the healthy group, one quarter/cow was chosen at random. The model building process continued with herd as a random effect.

All predictor variables in the initial model were screened for multicollinearity with correlation analysis using the Pearson correlation coefficients (continuous variables only) or the Spearman rank correlation coefficients. We defined the occurrence of multicollinearity at coefficients above 0.5. According to this definition, multicollinearity was present between milk yield and peak milk flow. To control for the multicollinearity between milk yield and peak milk flow, the peak milk flow was adjusted as described above. The model building process started with the baseline random intercept model to evaluate the random herd effect. Risk factors entered the base-line model separately to test whether the individual (fixed) factor affected the random herd effect. The final model was generated using stepwise backwards elimination from a full model including all risk factors. In each step, model quality was checked by the Log-Likelihood ratio test and by evaluation of Akaike's Information (Brown and Prescott, 1999). Coefficient estimates in different models were checked for changes indicating confounding or poor data quality. Risk factors tested in the initial models are described in Table 5.

The continuous variables DevPMF and herd prevalence were checked for linear relationship with the outcome variable by testing the model with polynomials of different degrees. Furthermore, linearity was tested by transforming the continuous variables in categorical variables. No first-order interactions between continuous variables were found. Interactions between continuous variables and categorical variables were not significant. Models including interaction terms between teat end shape, teat shape and teat orifice did not converge.

The random logistic regression model was as follows:

$$\text{Logit } P(Y)_{ijk} = \beta_0 + \sum \beta_i \text{RF}_i + \sum \beta_{jk} \text{FRF}_{jk} + \text{RH}_m \quad [1],$$

where P is the probability of Y (IMI with *Strep. uberis*, IMI with *Staph. aureus* or clinical mastitis), β_0 = intercept, β_i = regression coefficient for the continuous risk factor i , RF_i = value of risk factor i , FRF is the fixed effect of the categorical risk factor j on level k , RH_m = random herd effect for herd m .

The variances in the models were measured on the logit scale whereas the variance on residual level was measured on binomial scale. Under the assumption that no extra-binomial variation is permitted, level 1 (cow) variance was constrained to be equal to 1 on the binomial scale. The estimates of the proportion of variation of herd were computed by assuming that level 1 variance on the logit scale was $\pi^2/3$ (Dohoo et al., 2001).

RESULTS

During the study period 515 first lactation cows calved. Onehundredfiftyfive cows classified as ‘healthy’ were omitted from the analysis because their records covered less than 100 DIM. Seven cows had one blind quarter and were excluded from further analysis. Five of these cows left the herd in the first lactation, two in late lactation with mastitis as the main reason for culling.

During the first 100 DIM, 28 cows left the herd, mainly for breeding purposes (11 cows). Six cows were culled because of low milk yield, another six cows because of mastitis. The healthy group consisted of 232 cows. Eight of the 34 cows infected with *Strep. uberis* were analyzed in the clinical mastitis group because they showed clinical signs of mastitis before *Strep. uberis* was isolated. Five cows with IMI with *Strep. uberis* developed a clinical mastitis within the subsequent 14 days. The *S.-aureus*-group consisted of 28 cows and the clinical mastitis group of 51

cows. Fifty percent of the clinical cases occurred within the first week of lactation and 7 cows with clinical mastitis were infected with *Staph. aureus* within the next 28 days.

Descriptive statistics on cow and quarter level are shown in Table 3 and 4.

Table 3. Descriptive statistics primiparous cows, quarter level variables

Quarter level variables		<i>Strep. uberis</i> model		<i>Staph. aureus</i> model		Clinical mastitis model	
		Healthy n = 232	Strep. uberis n= 26	Healthy	S.aureus n = 28	Healthy	Clinical mastitis n = 51
Quarter	Frontquarter	110	17 (13.4)	109	15 (12.1)	108	35 (24.5)
	Hindquarter	122	9 (6.9)	123	13 (10.3)	124	16 (11.4)
Edema	Present	41	7 (14.6)	39	6 (10.3)	41	12 (22.6)
	Not present	191	19 (9.1)	193	22 (13.3)	191	39 (17.0)
Teat end shape	Inverted	9	2 (18.2)	10	3 (23.1)	8	4 (33.3)
	Flat	66	10 (13.2)	65	8 (11.0)	66	13 (16.5)
	Slightly rounded	153	14 (8.4)	153	15 (9.0)	154	34 (18.1)
	Rounded	4	0	4	2 (33.3)	4	0
Teat orifice condition	Teat canal protrusion	7	2 (22.2)	7	0	7	1 (12.5)
	Smooth callosity ring	118	7 (5.6)	118	12 (9.2)	120	20 (14.3)
	Rough callosity ring	15	5 (25.0)	16	2 (11.1)	16	8 (33.3)
	Normal orifice	92	12 (11.5)	91	13 (12.5)	89	22 (19.8)
Teat shape	Short and thin teats	24	3 (11.1)	25	2 (7.4)	23	0
	Short and thick teats	98	12 (10.9)	94	17 (15.3)	100	24 (19.4)
	Normal teats	68	8 (10.6)	71	5 (6.5)	70	11 (13.6)
	Conical teats	15	2 (11.8)	14	1 (6.1)	14	5 (26.3)
	Thick teats	27	1 (3.6)	28	3 (10.7)	25	11 (30.6)

Table 4. Descriptive statistics primiparous cows, cow level variables

Cow level variables		Healthy n = 232	<i>Strep.</i> <i>uberis</i> n= 26	<i>Staph.aureus</i> n = 28	Clinical mastitis n = 51
Calving season	Winter	85	18 (17.5)	15 (17.6)	34 (28.6)
	Summer	147	8 (5.2)	13 (8.1)	17 (10.3)
Breed	Angler	41	4 (8.9)	4 (8.9)	8 (16.3)
	Cross	3	1 (25.0)	3 (50.0)	3 (50.0)
	RH	98	9 (8.4)	16 (12.5)	15 (13.3)
	HF	90	12 (11.8)	7 (7.8)	25 (21.7)
Udder size	At hock	18	3 (14.3)	4 (18.2)	12 (40.0)
	Above hock	214	23 (9.7)	24 (10.2)	39 (15.4)
Milk leakage	Present	16	6 (28.6)	3 (15.8)	8 (34.8)
	Not present	216	20 (8.5)	25 (11.6)	44 (17.0)

Table 5 shows the results of a series of univariable random effects logistic regression models with herd as a random effect. The herd effect was statistically significant in all *Staph. aureus* models. The herd prevalence of *Staph. aureus* reduced the random herd effect considerably.

Important risk factors ($P \leq 0.1$) for IMI with *Strep. uberis* were milk leakage, DevPMF, condition of the teat orifice, calving in winter and herd prevalence of *Strep. uberis* in the preceding month. Teat end shape, having short and thick teats and herd prevalence of *Staph. aureus* in the preceding month were relevant risk factors for IMI with *Staph. aureus*. Important risk factors for clinical mastitis were udder size, milk leakage, teat orifice condition, hindquarter and calving in winter.

Table 5. Significance of independent variables in the univariable random effects logistic regression model with herd as random effect for the prediction of infections with *Streptococcus uberis*, *Staphylococcus aureus* or clinical mastitis during the first 100 DIM. Herd effect is given on the logit scale.

Independent variable	<i>Strep. uberis</i>		<i>Staph. aureus</i>		Clinical mastitis	
	Herd effect	P-value	Herd effect	P-value	Herd effect	P-value
Intercept	0.16		0.55		0.49	
Edema	0.20	0.174	0.60	0.345	0.49	0.602
Udder size	0.12	0.292	0.62	0.193	0.58	0.004
Milk leakage	0.34	0.020	0.52	0.584	0.48	0.140
DevPMF ¹	0.06	0.017	0.49	0.263	0.55	0.871
DevPMF (class)	0.11	0.251	0.52	0.526	0.49	0.351
Teat end shape (nominal)	0.18	0.455	0.61	0.110	0.49	0.429
Teat orifice condition (nominal)	0.01	0.184	Nc ²		0.46	0.111
Teat orifice condition (binary)						
Teat canal protrusion	0.15	0.676	Nc ²		0.41	0.565
Smooth callosity ring	0.02	0.082	0.56	0.456	0.41	0.101
Normal orifice	0.12	0.513	0.59	0.243	0.42	0.483
Rough callosity ring	0.10	0.102	0.55	0.995	0.45	0.049
Teat shape (nominal)	0.06	0.837	0.67	0.255	Nc ²	Nc ²
Teat shape (binary)						
Thick teats	0.11	0.388	0.57	0.797	0.48	0.014
Normal teats	0.14	0.473	0.58	0.170	0.42	0.262
Conical teats	0.15	0.882	0.65	0.620	0.40	0.627
Short and thin teats	0.12	0.632	0.60	0.498	Nc ²	Nc ²
Short and thick teats	0.15	0.722	0.68	0.032	0.39	0.890
Calving in winter	0.26	0.009	0.58	0.144	0.42	0.002
Breed	0.53	0.351	0.82	0.114	0.31	0.487
Rear quarter	0.32	0.261	0.56	0.423	0.50	0.023
Day of udder examination	0.27	0.053	0.86	0.002	0.42	0.022
Herdprevalence CPS (class)			0.0	0.032	0.62	0.482
Herdprevalence <i>Strep. uberis</i>	0.10	0.012	- ³	- ³	0.52	0.512
Herdprevalence <i>Strep. uberis</i> , squared					0.47	0.071

¹Deviation from mean peak flow rate adjusted for milk yield in kg/min.

²Model does not converge.

³Not tested.

Results from the multivariable models

IMI with *Strep. uberis*

DevPMF was a significant risk factor for IMI with *Strep. uberis* (Table 6). The mean DevPMF for healthy cows was 0.04 ± 0.86 kg/min whereas cows with *Strep. uberis*-infection had a mean DevPMF of 0.45 ± 0.93 kg/min. Calving in winter and teat canal protrusion as well as rough teat end callosity were significant risk factors for IMI with *Strep. uberis*.

Table 6. Significant risk factors for *Strep. uberis* infections in primiparous cows in a random logistic regression model. The parameter estimates (b), standard error for the estimates (SE), odds ratio (OR), 95% confidence interval (CI) for OR and significance level from F-test are given for each risk factor.

Risk factor	Level	b	SE	OR	95% CI for OR	P
Intercept		-2.41	0.66			
Calving in winter		1.43	0.46	4.2	1.7 – 10.4	0.009
DevPMF ¹		0.73	0.25	2.1	1.3 – 3.4	0.004
Teat orifice condition	Protrusion	2.0	1.0	7.7	1.1 – 52.6	0.006
	Smooth	-0.6	0.5	0.54	0.2 – 1.4	
	callosity					
	Rough	1.8	0.7	6.1	1.5 – 24.2	
	callosity					
	normal	0		1		
Day of udder examination		-0.04	0.01	0.96	0.94 – 0.98	0.005
Herd prevalence of preceding month		0.34	0.15	1.4	1.0 – 1.9	0.025

¹Deviation from mean peak flow rate adjusted for milk yield in kg/min.

The random herd effect was low and only contributing with 2% of the variance (Table 9). When herd prevalence of *Strep. uberis* entered the model, the random herd effect dropped to zero. There was a linear relationship between herd prevalence of *Strep. uberis* and the risk of infection with *Strep. uberis*.

IMI with *Staph. aureus*

The herd prevalence was the most important risk factor for IMI with *Staph. aureus* (Table 7). The relationship was non-linear and showed a linear increase up to a prevalence of 2% and a curvilinear slope with higher herd prevalences. Therefore herd prevalence was specified in the

model as categorical variable. When herd prevalence entered the model, the random herd effect decreased from 29.4% to 6%. Cows with short and thick teats as well as cows with pointed teats were at higher risk of infection with *Staph. aureus*.

Table 7. Significant risk factors for *Staph. aureus* infections in primiparous cows in a random logistic regression model. The parameter estimates (b), standard error for the estimates (SE), odds ratio (OR), 95% confidence interval (CI) for OR and significance level from F-test are given for each risk factor.

Risk factor		b	SE	OR	95% CI for OR	P
Intercept		-2.52	0.79			0.001
Herdprevalence of preceding month	> 2%	2.70	0.81	14.9	3.1 – 72.4	0.010
	-2 % 0	2.37	0.74	10.7 1	2.48 – 47.0	0.013
Short and thick teats		1.04	0.34	2.8	1.3 – 6.1	0.018
Teat end shape	Inverted	0.79	0.71	2.2	0.5 – 8.9	
	Flat	0.11	0.42	1.1	0.5 – 2.6	
	Pointed	2.49	0.85	12.1	2.3 – 63.6	0.007
	Round	0		1		
Day of udder examination		-0.04	0.01	0.98	0.97 – 1.00	0.001

Clinical mastitis

From calving to 100 DIM 51 primiparous cows experienced at least one episode of clinical mastitis. Clinical mastitis was not observed among the 23 cows with small and thin teats and in four cows with pointed teat ends (Table 3). Cows with udders at hock level and long and thick teats were at higher risk of clinical mastitis. There was confounding present between milk leakage and calving in winter. Cows calving during the winter season were more likely to have milk leakage than cows calving during the summer months. The herd prevalence of *Strep. uberis* was a significant risk factor in the univariable model, but confounding was observed with calving season. Because management during calving season influences the infections risks and herd prevalence, calving season was kept in the model process. The random herd effect accounted for 16.7% of the variance in the data (Table 9).

Table 8. Significant risk factors for clinical mastitis in primiparous cows in a random logistic regression model. The parameter estimates (b), standard error for the estimates (SE), odds ratio (OR), 95% confidence interval (CI) for OR and significance level from F-test are given for each risk factor.

Risk factor	b	SE	OR	95% CI	P
Intercept	-2.34	0.56			
Calving in winter	1.21	0.36	3.4	1.7 – 6.8	0.004
Hindquarters	0.87	0.345	2.4	1.2 – 4.7	0.024
Udder at hock level (vs. above)	1.07	0.44	2.9	1.2 – 7.1	0.034
Long and thick teats	0.71	0.39	2.0	0.95 – 4.4	0.089
Day of udder examination	-0.01	0.01	0.99	0.98 – 1.00	0.160

Table 9. Estimates of variance components (b) on logit scale, standard error (SE), significance level from Wald's test and proportion of variance explained at each level of the hierarchy in the *Staph. aureus*, *Strep. uberis* and clinical mastitis model.

Model	Herd				Residual (cow)			
	b	SE	P	Proportion (%)	b	SE	P	Proportion (%)
<i>Strep. uberis</i>	0.08	0.19	0.33	2	0.87	0.06	<0.0001	98
<i>Staph. aureus</i>	0.21	0.83	0.287	6.0	0.70	0.06	<0.001	94
Clinical mastitis	0.67	0.43	0.06	16.7	0.90	0.08	<0.001	83.3

DISCUSSION

IMI with *Staph. aureus*

As expected, the herd prevalence of quarters shedding *Staph. aureus* was an important risk factor and 'explained' the random herd effect. The risk for infection did not increase linearly with herd prevalence. One reason may be changes in management to reduce the number of new infections. Culling or treatment of infected cows could have reduced the newinfectionrate and the herd prevalence. Milking hygiene practices were not specified as fixed effects in the analysis because the cause – effect chain is not clear. Milking practices such as teat dipping or cluster disinfection between cows may be carried out or changed according to the current infection status.

A further problem is the consistency in application of control methods that can differ between the farm visits.

Short and thick teats were at higher risk for IMI with *Staph. aureus*. When short and thick teats are milked, they will in general not penetrate beneath the collapsing point of the liner. As a result, the liner cannot compress the teat end and decrease fluid accumulation in the teat end. The resulting teat damage and disturbance in circulation could have facilitated the penetration and manifestation of *Staph. aureus*. A negative effect on udder health by hard and swollen teats has been reported by Rasmussen (1998) and Rønningen and Reitan (1990). In our study, short and thick teats had a 2.2 times higher risk of being hard or swollen after cluster removal (24% in cows with short and thick teats vs. 13% in all other teat shapes, $P < 0.05$ chi square test). In contrast to Bakken (1981) we did not find a relation between *Staph. aureus* and conically shaped teats.

IMI with *Strep. uberis*

The risk for IMI with *Strep. uberis* increased linearly with increasing DevPMF. Cows with higher DevPMF may be less resistant to IMI because of a wider teat canal diameter (McDonald, 1975). DevPMF differed widely among farms (-0.8 to 0.45), which should be taken into account when a herd specific mastitis prevention program is developed. The higher risk for infection during the winter period may reflect the higher concentration of *Strep. uberis* in the bedding and the hygiene in the barn due to higher stocking rates in comparison to the summer period when all heifers and cows were on pasture. Another aspect may be increased level of stress during the housing period in the winter months because of reduced space per cow. O'Connell et al. (1989) found more agonistic and less grooming behaviour in confinement housed cows in comparison to cows on pasture. The increased level of stress may especially have affected primiparous cows because of their lower rank.

In contrast to Zadoks et al. (2001) we found a higher risk of *Strep. uberis* in cows with a rough teat end callosity. The rough teat end callosity may have disturbed local defense mechanisms. Furthermore, the presence and growth of bacteria may be enhanced in a cracked surface and manure particles are difficult to remove with teat preparation before milking. The herd effect was negligible, indicating that risk factors were cow related and that there is little difference in risk present between the studied herds.

Clinical mastitis

Consistent with other reports (Seykora and McDaniel, 1985, Slettbakk et al., 1995), cows with deeper udders were at higher risk for clinical mastitis. Cows with deeper udders may have produced more milk (Slettbakk et al., 1990, Rogers and Spencer, 1991) and the high milk yield may have increased the risk for clinical mastitis. Because 50% of the clinical mastitis cases occurred within the first week of lactation and before milk recording was carried out, the effect of milk yield on the risk of clinical mastitis could not be estimated in our dataset.

Deeper udders may lead to heavier contamination with environmental pathogens as well as they may hinder the cow when lying down and increase the risk for teat lesions. Because teat orifice condition changes especially in the first weeks of lactation (Neijenhuis et al., 2000) and clinical mastitis may have affected milking ease, teat orifice condition was omitted from the final model.

DIM at udder examination was forced into the model for clinical mastitis because DIM may have affected teat and udder shape. Teat length increases throughout lactation in first lactation cows (Binde and Bakke, 1984). Larger teats may have a wider teat canal or a wider stretchable teat orifice as reported by (Seykora and McDaniel, 1985) that facilitate penetration of bacteria. Teats with wide diameter are associated with increased liner slips (Rogers and Spencer, 1991). The authors also found an increased number of liner slips in deeper udders. Liner slips cause irregular vacuum fluctuations under the teat end that can lead to large impact forces on the teat ends (O' Shea et al., 1975). Especially when impacts occur at the end of milking the penetrating bacteria may remain in the gland.

DevPMF was not associated with the risk of clinical mastitis. It has to be considered that the clinical cases often occurred before the first milk recording and the first recording of peak milk flow. Fibrous tissue or scar tissue may have remained and lead to a lower PMF in cows that had experienced clinical mastitis, even after the infection had been eliminated.

The results of the three models have to be interpreted with caution due to the low number of affected animals. Clinical mastitis and infections with *Strep uberis* occurred more often during the housing period. A preventive effect of pasture was also observed by Washburn et al. (2002). Cows with short and thin teats never had clinical mastitis, probably due to a lower milk

yield in these cows. We would expect a higher infection risk in these cows because of the longer milk duration induced by the small teat canal. But the tight closure seems to prevent infection.

In contrast to Waage et al. (2001), we could not find an effect of udder edema and milk leakage on the risk for CM. An explanation may be the difference in recording: Waage and colleagues asked the farmers for information on edema and milk leakage at calving whereas in our study edema was assessed on the day of the farm visit. In another study periparturient edema was a significant risk factor for clinical mastitis whereas no effect of milk leakage between milkings (farmers observation) could be shown (Slettbakk et al., 1995).

Our results show that different udder and teat characteristics are risk factors for IMI with *Strep. uberis* and *Staph. aureus*. This could explain why some authors find an association between SCC and teat characteristics while others do not. The prevalence of major pathogens in the studied herds may have been different. Furthermore, there are differences in risk factor patterns between a quarter becoming infected and a quarter developing clinical signs of mastitis. This should be taken into account when mastitis control measures are implemented in farm.

DevPMF was only significant for IMI with *Strep. uberis*. The relation between mastitis and peak milk flow is controversial in literature. Some authors find a relationship between SCC and maximum milk flow or 2-min milk (Slettbakk et al., 1990) When comparing these studies, the definition of mastitis, clinical or subclinical, SCC, and bacteriology have to be considered as well as the adjustment of the correlation between milk yield and milk flow and stage of lactation.

Herd effect

Random herd effect in general was low in all models. However, random herd effect was highest in the *Staph. aureus* model, before prevalence of infected quarters entered the model. Without herd prevalence as fixed effect in the model, random herd effect accounted for 29.4% of the variation in the data. When herd prevalence entered the model, variation due to herd effects was reduced to 6%, i.e. herd prevalence accounted for approximately 80 % of the variation on herd level. The same happens when herd prevalence enters the *Strep.-uberis*-model: the variation explained by herd went down from 2% to zero.

In all models most of the variation was on cow level, even in the *Staph. aureus*-model without considering herd prevalence. One reason could be that the variation between herds regarding system and management practices was low. All herds already applied hygiene practices to

combat contagious pathogens: All cows were dried off with long acting antibiotics and 14 herds practiced teat dipping after milking. Housing systems were quite similar, at least with regard to the lactating cows and grazing practices. The herd prevalence of *Staph. aureus* can be used as a measure for the success of the implemented mastitis control strategies in a herd. A reduction of the herd prevalence of *Staph. aureus* will reduce the risk for primiparous cows considerably.

CONCLUSION

This study indicates that different udder and teat characteristics are risk factors for IMI with *Strep. uberis* and *Staph. aureus* and cases of clinical mastitis. Further large-scale studies are necessary to assess the potentials of using pathogen specific udder and teat characteristics in mastitis control and breeding plans.

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Due to restrictions from the publisher of the journal in which manuscript 1 and 3 have been published, these articles are not available in this PDF. The articles can be found in:

Manuscript 1:

Klaas, I. C., Enevoldsen, C., Vaarst, M., & Houe, H. (2004). Systematic Clinical Examinations for Identification of Latent Udder Health Types in Danish Dairy Herds. *Journal of Dairy Science*, 87, 1217-1228.

Abstract:

Milk leakage in dairy cows is a symptom of impaired teat sphincter function. Milk leakage is related to an increased risk of mastitis in heifers and cows, and causes hygiene problems. The aim of our study was to assess whether teat shape, condition of teat orifice, and peak milk flow rate are risk factors for milk leakage. We conducted a longitudinal observational study in 15 German dairy farms in which cows were maintained in loose housing. The farms were visited monthly at 2 consecutive milkings. During the evening milking, milk flow curves were measured with the LactoCorder. Milk leakage was recorded during the subsequent morning milking, when cows entered the milking parlor. Immediately after detachment of the milking cluster, teat shape, teat end shape, and condition of the teat orifice of cows were assessed between 9 and 100 d in milk (DIM) and during late lactation (>250 DIM). Data from 1600 cows were analyzed. Milk leakage was treated as the binary response variable in a logistic regression model with herd as a random effect. Primiparous cows with high peak milk flow and teat canal protrusion were at greater risk of milk leakage. High peak milk flow rate, short teats, teat canal protrusion, inverted teat ends, and early lactation increased the risk of milk leakage in multiparous cows. Random herd effects accounted for only 10% of the total variation, indicating that the impact of management or other herd-level factors on the occurrence of milk leakage is virtually negligible for practical purposes.

Abbreviation key: **DevMilk** = deviation from the population mean milk yield adjusted for stage of lactation and parity, **DevPMF** = deviation from the population mean peak milk flow adjusted for stage of lactation and parity, **MeanResMilk** = mean residual of observed –expected milk yield

Manuscript 3:

Klaas, I. C., Enevoldsen, C., Ersboll, A. K., & Tolle, U. (2005). Cow-Related Risk Factors for Milk Leakage. *Journal of Dairy Science*, 88, 128-136.

Abstract:

A cross-sectional study was conducted to explore the applicability of systematic clinical examinations of udders as an additional tool for the evaluation of udder health status on dairy farms. During 2000, each of the 16 dairy farms was visited 5 times; 20 cows per farm were chosen at random at each visit for clinical udder examination immediately after milking. The clinical examination included both pathological and morphological variables. One examination per cow was included in the analysis (n = 707 cows). Principal component analysis (PCA) was performed in 3 steps. First, 19 variables characterizing udder and teats were analyzed (PCA 1). Second, the variables parity and stage of lactation were included (PCA 2). Finally, somatic cell count (SCC) and milk yield (PCA 3) were included. The PCA resulted in 4 components that explained 30% of the variation of the data: 1) small udder, 2) distressed udder, 3) mastitis udder, and 4) soiled udder. Variables with high positive correlation to the "small udder" were small udder shape, short teats, and first parity. Impaired teat surface, hard udder texture, and a long udder shape were related to the "distressed udder." The "mastitis udder" was characterized by the clinical variables asymmetry between front quarters, asymmetry between hind quarters, knotty tissue, and acute clinical mastitis. Reduced milk yield and high SCC were related to the "mastitis udder," whereas low SCC was related to the "small udder." The "soiled udder" was related to early lactation. Including this information in the assessment of udder health may be of substantial value for data analysis in farms with suspected under-reporting of clinical mastitis.

Abbreviation key: **ECM** = energy-corrected milk, **MLOGSCC** = mean of log-transformed SCC, **MRESMILK** = mean residual of observed-expected milk yield, **PCA** = principal component analysis

Udder health indicators and veterinary treatments in organic dairy herds with a non-antibiotic treatment strategy

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Abstract

The aim of our study was to assess udder health by using systematic clinical examinations in 23 organic dairy herds that wanted to phase out antibiotic treatments. Somatic cell count (SCC) and records of treated cases of mastitis may be insufficient to document udder health and animal welfare. The udders were clinically examined during milking at the beginning of the study period. The hygiene of body and udder was scored. The data was analysed using principal component analysis and a general linear model. The prevalence of cows with nodes in the udder was 0.07 and the prevalence of cows with a dry quarter was 0.08. Danish Holsteins had more veterinary-treated cases of mastitis than Jerseys. Principal component analysis extracted two components: component 1 was characterized by udder and body hygiene scores, mastitis treatments and Danish Holsteins, whereas component 2 was characterized by nodes in the udder, knotty tissue and dry quarters. Farms loading high on the components had a higher calculated bulk milk SCC. Nodes, knotty tissue and blind quarters were not associated with the number of udder treatments. This indicates that a lower treatment frequency did not lead to a higher proportion of cows with chronic mastitis. Systematic clinical udder examinations were useful tools to monitor udder health.

Key words: organic farm, mastitis treatment, udder characteristics, hygiene

Introduction

An important goal of organic farming is the promotion of animal health and a reduced usage of antimicrobial agents. In organic dairy farming, mastitis is the most important disease regarding the amount of antibiotics used for treatment (Bennedsgaard 2003). The risk of antibiotic resistance, poor mastitis treatment results and the concerns of the consumers regarding antibiotic residues in milk products have started a discussion among Danish organic farmers to promote explicit non-antibiotic treatment policies in their herds. As a result of these discussions, a project was initiated in 2004 with the aim to phase out antibiotic treatments in 23 organic farms. A non-antibiotic treatment strategy could compromise animal welfare, if farmers to a smaller or larger extent just stop calling the veterinarian, thereby leaving the cows untreated. The farmer's decision to treat a cow with mastitis with antibiotics depends on the severity of the symptoms, the characteristics of the cow, the infection level in the herd and his attitudes towards alternative treatment (Vaarst and others, 2002). All participating farmers expressed that they wanted to keep the opportunity for antibiotic treatments in cases of emergency, but their thresholds when regarding a case as emergency may differ. Under an explicit non-antibiotic treatment strategy, the number of treated cases of mastitis does not reflect the true number of mastitis cases; the treated cases will rather reflect the farmer's mastitis management. Depending on the farmer's attitude towards alternatives to antibiotic treatments, they may choose to blind infected quarters instead of treating quarters with antibiotics in order to control somatic cell counts. To assess udder health in such herds, monitoring of cow somatic cell counts and treated clinical cases may not be sufficient. Consequently, systematic clinical examinations of the udders may be an important tool to assess udder health in organic farms with a non-antibiotic treatment strategy. Klaas and others (2004a) demonstrated that the mastitis udder was characterized by asymmetric quarters and high somatic cell count. The prevalence of dry quarters, asymmetric quarters or in general quarters with pathological changes may give us important information about the udder health status and mastitis management in farms with a non-antibiotic treatment policy. Udder and leg hygiene scores were associated with SCC and are regarded as important risk factors for mastitis (Schreiner and Ruegg, 2003). The susceptibility of mastitis may differ among breeds. In an experimental study Jersey cows showed less cases of clinical mastitis than Holstein cows (Washburn and others, 2002).

The objective of the present study was to explore the relation between indicators for mastitis on herd level from different sources of information, including the results from systematic

clinical examinations. Furthermore, we wanted to evaluate, whether an explicit non-antibiotic policy had a negative effect on udder health.

Material and Methods

Twentythree organic farms participated in a project with the aim to phase out antibiotic treatments. The farms were located in central and northern Jutland in Denmark and delivered milk to the Thise Dairy. In 13 farms cows were kept on deep litter, 10 farms had free stalls with concrete or slatted floor. Thirteen farms had Danish Holstein cows (DH), the other 10 kept mainly Jersey cows or cross-breds. In Danish organic farms, all antibiotic treatments are carried out by the veterinarian. All veterinary treatments are reported to and stored in the Danish Cattle Database. Herd characteristics are presented in Table 1.

TABLE 1. Herd characteristics of the 23 organic dairy herds measured during the year before the study period

Variables	Mean (se)	Min	Max	Interquartile range
Herd size (no. cows)	73.7 (33.2)	34	158	41
Calculated BSCC ¹ x1000/ml	266 (90.4)	93	446	139
305 day ECM ² , kg	6501 (911)	4880	7930	1528
Mastitis treatments per cow year ³	0.16 (0.15)	0	0.48	0.20
SCC rank of culled cows ⁴	0.69 (0.08)	0.53	0.89	0.09

¹ Herd mean calculated bulk somatic cell count (x1000/ml) from milk recordings 12 months prior to the clinical examination.

²Energy corrected 305 day milk of first parity cows (herd mean)

³Within 12 months prior to the clinical udder examination.

⁴At the last test-day before culling. High values indicate a high probability that the cow was at least partly culled due to high somatic cell count.

At the start of the project in March and April 2004, the first author examined the udders of all cows immediately after milking. In farms milking more than 50 cows, a random sample of 50 cows was examined.

The indicators for mastitis chosen in this analysis are defined below. To account for seasonal effects, production data and veterinary treated cases of mastitis covered a period of one year

prior to the systematic clinical examination. Mean DIM was the herd average days in milk at the clinical examination.

Indicators based on routinely recorded data

Calculated bulk somatic cell count (CBSCC) was the herd mean calculated bulk somatic cell count ($\times 1000/\text{ml}$) from milk recordings 12 months prior to the clinical examination.

Mastitis treatments were the veterinary treated cases of mastitis per cow year within the last 12 months prior to the clinical examination.

SCC rank of culled cows was the average ranking of the culled cows within her herd mates according to the SCC from the last test day before culling.

ECM305 was the mean energy corrected milk yield of all first parity cows within a herd. Milk yield was adjusted for breed using a general linear model.

Clinical indicators

Dry quarters were all quarters that were not milked at the day of clinical examination. The diagnosis was based on observation (asymmetry, condition of teat) and palpation (obliteration of teat canal, atrophic tissue, nodes).

Knotty tissue was diagnosed when more than 5 small nodes of less than 2 cm were palpable. To differentiate between coarse udder texture and pathologic changes, all quarters were compared for differences.

Nodes were distinct nodes of at least 2 cm in diameter or the whole quarter appeared as one hard structure.

Asymmetric quarters were recorded when asymmetry occurred between front quarters or between hindquarters.

The prevalence of dry quarters, nodes, knotty tissue or asymmetric quarters was the number of cows with at least one affected quarter divided by the number of cows examined.

Hygiene scores

The degree of soiling with manure on the hind legs and body was scored from 0-4 with 0=no soiling up to 4=large part of the body is covered with manure. The degree of soiling of the udder was scored from 0-2 with 0=no soiling up to 2 = manure covers a larger part of the udder. The side with the worst condition was recorded. Herd leg hygiene and herd udder hygiene scores were the herd mean of degree of soiling.

All indicators were analysed on herd level.

Statistical analysis

The various indicators chosen to describe udder health may be closely related to each other and they may be insufficient to describe udder health status as single variables. Principal component analysis (PCA) is a technique that removes multicollinearity and provides insight into how the variables are related to each other (Dohoo and others, 1996 and 2003). The information contained in all predictor variables is consolidated into a set of uncorrelated predictor variables (= components). PCA attempts to identify a minimum number of components explaining the majority of the variation in the original data. We used the factor procedure of SAS (1999). Components were retained when they had eigenvalues greater than one, i.e. the component explained more variation in the data than the individual variable alone, and the component was interpretable in a meaningful way (Hatcher, 1994). Further, a component was regarded as important if at least three variables had high loadings on that component and shared the same conceptual meaning. A factor loading greater than ± 0.4 was considered to be important. Loadings range from -1 to $+1$ and represent the correlation of a given variable with the underlying factor. According to the criteria stated above and supported by the scree test that displayed two components with the largest eigenvalues before a visible 'break', two components were retained.

The relation between breed and mastitis treatments per cow year was examined using a Wilcoxon test. Milk yield from the monthly milk recording was transformed in energy corrected milk (Sjaunja and others 1990). The lactation curve was estimated with a piecewise linear regression model at cow level (Klaas and others 2004a). The herd's deviation from the mean ECM305 of first parity cows within breed was included in the PCA.

Two general linear regression models (proc GLM, SAS 8.2) were used to analyse the relation between the extracted components and CBSCC as outcome variable and the relation between the individual variables and CBSCC. The variables herd leg hygiene score, herd udder hygiene score, prevalence of nodes prevalence of nodular tissue, prevalence of dry quarters prevalence of asymmetric quarters, Danish Holstein and mastitis treatments per cow year entered the model one at a time and subsequently in a model building strategy based on backward elimination.

The relation between the extracted components and CBSCC was visualized with a smoothed scatter plot, using local regression techniques (proc GAM, SAS 8.2, 1999).

Results

The organic farms had on average 0.16 mastitis treatments per cow year (Table 1); six farms did not have any antibiotic mastitis treatments during the year before the study. Farms keeping DH had more mastitis treatments per cow year than farms with Jerseys or cross breeds (0.23 vs. 0.07, $P < 0.01$). In three farms no cow with a dry quarter was found whereas in other farms up to 18% of cows had a dry quarter (Table 2). Leg hygiene scoring showed that 18.2% of the cows had completely clean legs (score 0), 63% had spots with manure at the lower leg (score 1). Only 2 cows had the whole body covered with manure (score 4). 73% of the udders were completely clean, 24% had spots of manure on the udder and 3% had larger parts of the udder covered with manure. Three farmers had more than 0.40 mastitis treatments per cow year, two of them with less than 5% cows with dry quarters.

TABLE 2. Herd level results from the systematic clinical examinations in 23 organic dairy herds

Variables	Mean (se)	Min	Max	Interquartile range
Herd leg hygiene score ¹	1.30 (0.40)	0.35	1.85	0.48
Herd udder hygiene score ²	0.28 (0.22)	0	0.83	0.37
Prev. of nodes ³	0.07 (0.06)	0	0.19	0.09
Prev. of nodular tissue ⁴	0.17 (0.13)	0	0.49	0.19
Prev. of dry quarters ⁵	0.08 (0.06)	0	0.19	0.10
Prev. of asymmetric quarters ⁶	0.14 (0.09)	0	0.35	0.08
Mean parity	2.52 (0.38)	2	3.6	0.5
Mean DIM	182 (47)	115	301	46

¹Herd mean of degree of soiling with manure on legs scored from 0-4. 0=no soiling, 4=whole body covered with manure

²Herd mean of degree of soiling with manure on udder scored from 0-2. 0=no soiling, 2 = manure on larger parts of the udder

³Prevalence of cows with distinct nodes in at least one quarter

⁴Prevalence of cows with knotty tissue in at least one quarter

⁵Prevalence of cows with dry quarters, cows that did not produce milk in 1 quarter

⁶Prevalence of cows with slight or pronounced asymmetry between front- or between hindquarters

The PCA extracted 2 components that explained 64% of the variation in the data (Table 3). Component 1 was labelled ‘Soiling, DH, asymmetric quarters & treatments’ because it

consisted of high correlation coefficients of the variables dirty leg score, dirty udder score, DH, asymmetric quarters and mastitis treatments. Herds loading high on component 1 had a lower mean DIM at the day of the clinical examination. Component 2 was characterized by the prevalence of cows with nodes, prevalence of cows with a dry quarter, and prevalence of cows with knotty tissue, which were interpreted as variables that describe an udder with chronic clinical changes and therefore labelled ‘chronic mastitis udder’. Based on the PCA we could not detect a significant correlation or pattern between mastitis treatments per cow year and the prevalence of cows with dry quarters.

TABLE 3. Principal components analysis: Correlations (standardized regression coefficient) between udder health parameters and 2 components after orthogonal rotation (varimax rotation).

	Component 1 ‘Soiling, DH & treatments’	Component 2 ‘Chronic mastitis udder’
Herd leg hygiene score	0.88 *	-0.15
Herd udder hygiene score	0.85 *	-0.06
Danish Holstein (DH)	0.82*	0.03
Mastitis treatments per cow year	0.71*	0.24
Prevalence of asymmetric quarters	0.68*	0.28
Mean DIM	-0.60*	-0.19
Prevalence of cows with nodes	0.01	0.85*
Prevalence of dry quarters	0.04	0.84*
Prevalence of cows with knotty tissue	-0.04	0.78*
Eigenvalue	3.51	2.10
Variance explained (%)	39	24

* correlation coefficients > 0.4 were regarded as significant

The relationship between the extracted two components and the CBSCC was examined in a linear regression model. Both components showed a significant association with CBSCC that explained 48% of the variation between the farms on CBSCC (Table 4). An increase of component 1 of 1 unit was associated with an increase of CBSCC by 46.

TABLE 4. Results from the linear regression model: the effect of the two components on CBSCC, $r^2 = 0.48$

	Estimate	Std	P-value
Intercept	300.9	20.2	<.0001
Component 1	46.9	15.1	0.0049
Component 2	46.0	16.3	0.0110
Component 2 squared	-36.2	14.5	0.0221

Component 2 showed a non-linear relationship with CBSCC as demonstrated in Figure 1. CBSCC increased with component 2 for negative scores, but for scores above 0 no relation was seen. Figure 2 shows the relation of the 2 components on CBSCC and the contribution of mastitis treatments. CBSCC is cut in three categories. The lowest CBSCC was observed in farms loading high on component 1 and low on component 2. Farms with negative loadings on both components had medium CBSCC between 200,000 and 300,000; only one had a CBSCC less than 200.

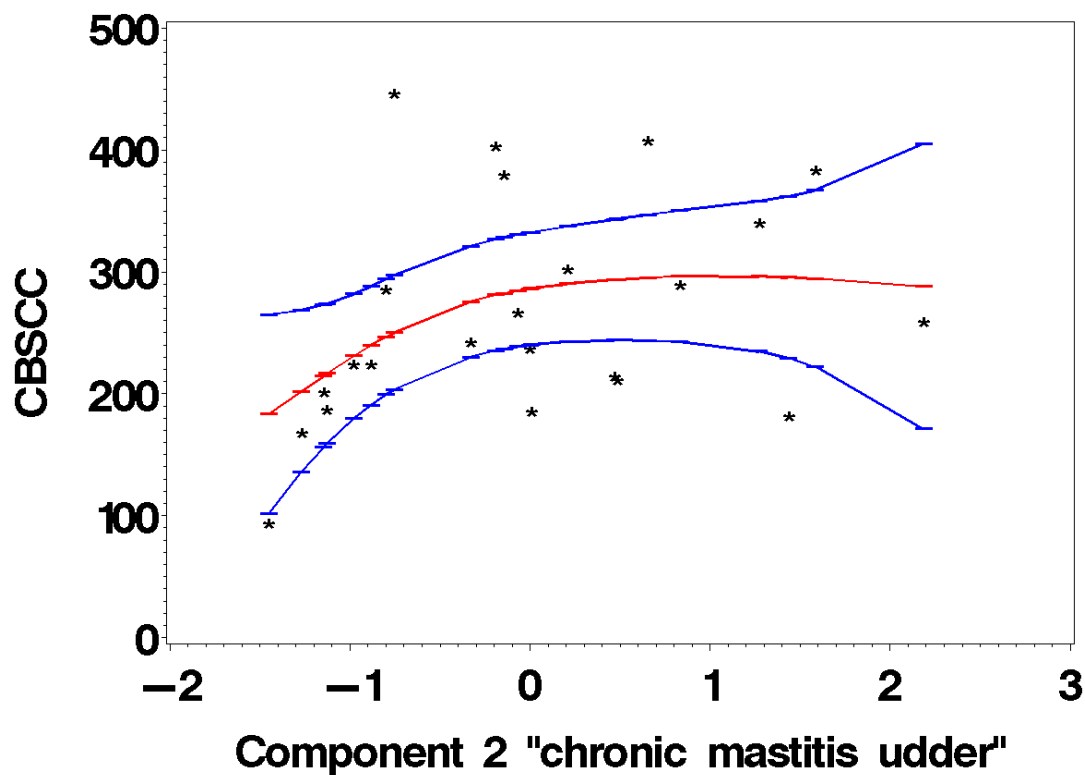


FIG 1. Relation between component 2 and calculated bulk SCC (CBSCC, in 1,000/ml). Smoothed scatterplot with tendency line (red) and 95% confidence limits (blue).

The results of the PCA were compared to a model for CBSCC where all variables entered the model individually (TABLE 5). Increasing herd leg hygiene score and prevalence of cows with nodes were associated with an increase of CBSCC and explained 44% of the variation between herds on CBSCC. All other variables were not significant in the multivariable model.

TABLE 5. Results from the linear regression model: the effect of the variables on CBSCC, $r^2 = 0.44$

	Estimate	Std	P-value
Intercept	99.3	46.1	0.0436
Herd leg hygiene score	117.5	38.4	0.0062
Prev. of cows with nodes ¹	6.9	2.6	0.0148

¹Increase of 1 unit = 1%

Discussion

The organic farmers had on average 0.16 mastitis treatments per cow year, which is very low in comparison to 0.33 mastitis treatments in 24 Danish organic herds converted before 1990, and 0.50 mastitis treatments in organic herds that converted in 1995 (Bennedsgaard and others 2003). Conventional Danish herds had 0.59 mastitis treatments per cow year (Bennedsgaard and others, 2003). But despite a low average of mastitis treatments, farms varied considerably indicating that the farmers already were in different stages towards their goal of phasing out antibiotic treatments, even before the study period. Six farms with no antibiotic mastitis treatments already had reached their goal at the beginning of the study.

We expected that our farms with the goal to phase out antibiotics might have had a higher proportion of cows with dry quarters. On average 8% of the cows had a dry quarter, which is comparable to the results of Houe and others (2002) in 4 conventional herds and Klaas and others (2004b) in 8 farms with automatic milking system. Drying off a single quarter can be regarded as alternative to antibiotic treatment for cases of chronically high SCC or recurrent mastitis (Vaarst and others, 2002). Three of our study farmers had at least 14% of cows with dry quarters, whereas three farmers did not have any cows with dry quarters at the clinical examination, which may reflect two types of attitudes as described by Vaarst and others (2002). The authors interviewed conventional farmers and detected two distinct groups regarding the practice of drying off quarters as treatment alternative. One group of farmers

practiced drying off single quarters whereas the other group regarded having cows with dry quarters as bad management. A short term experimental study (Hamann and Reichmuth 1990) showed that the milk yield of the other 3 milked quarters increased by 4%. So there is economic loss because of reduced milk yield, but the effect is partly compensated by increased production in the remaining quarters.

We used SCC rank of culled cows at the time of culling to have a measure for mastitis as culling reason independent on the farmers' priorities in culling management, but we could not detect a relationship to the other udder health indicators and the two extracted components. Valde and others (2005) did not find any relationship between cows culled due to mastitis and udder health parameters from the milk recording.

Poor hygiene, DH, asymmetric quarters and high incidence of udder treatments were related. There are several explanations to be discussed. Dirtier legs and udders reflect a higher risk of getting mastitis. Secondly, farmers that do not focus on keeping their cows clean may be more likely to treat mastitis cows with antibiotics. Cows in early lactation were associated with higher degree of soiling of the udder (Klaas and others, 2004a). We used mean DIM to adjust for seasonal calving patterns. The negative correlation between mean DIM and component 1 indicates that farms loading high on component 1 did not have seasonal calving and that having more cows in early stages of lactation is related to having dirtier cows. DH had a higher milk yield than Jerseys. Milk yield was loading significantly on component 1 in the initial PCA, but when milk yield was adjusted for breed, the adjusted milk yield did not show significant loadings on any of the two components and therefore was excluded from the PCA, while breed effect still remained important.

Our hypothesis was that farms with a non-antibiotic treatment strategy might have had a higher proportion of cows with chronic changes as expressed with high loadings on component 2. Figure 2 shows an even distribution of farms within the four quadrants. This indicates that a low usage of antibiotic treatments of mastitis did not affect the number of cows with chronic changes in the udder.

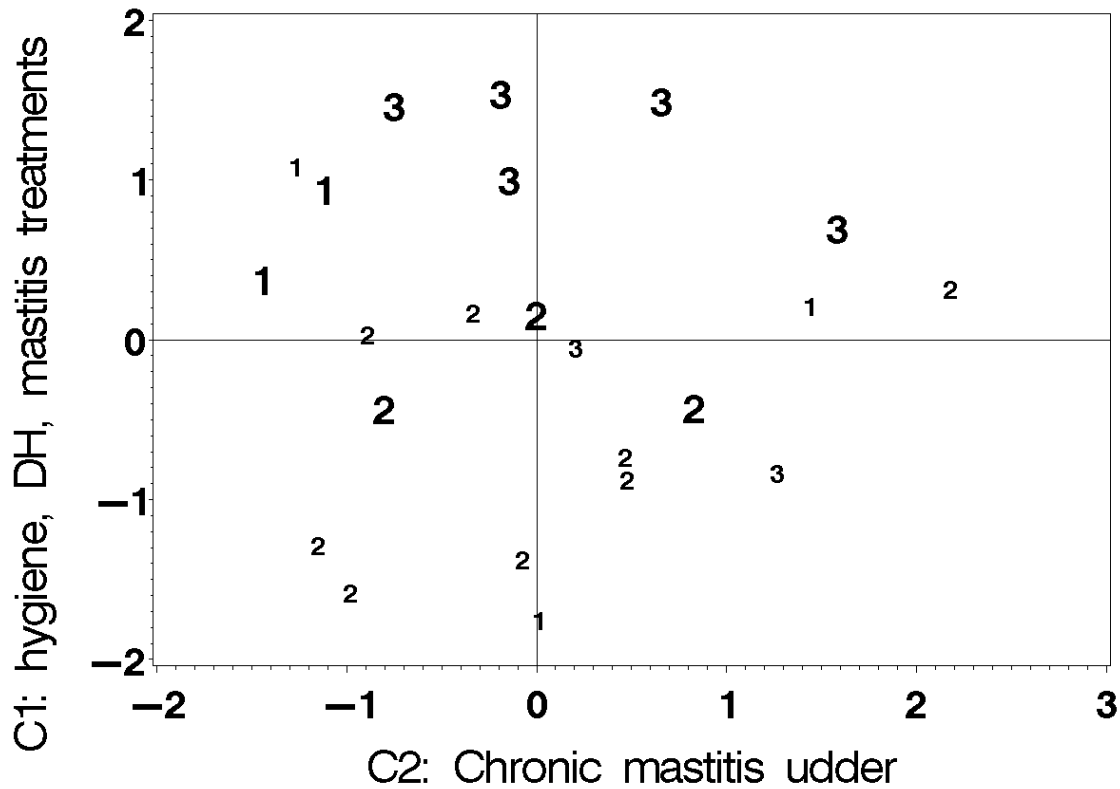


FIG 2. Relation between the 2 principal components and the calculated bulk tank SCC. Component 1: Soiling, DH & treatments, Component 2: Chronic mastitis udder. CBSCC (in 100/ml) 1: < 200, 2: 200-300, 3: ≥ 300 . Numbers in regular font: < 0.16 mastitis treatments per cow year. Boldface numbers: ≥ 0.16 mastitis treatments per cow year.

The comparison of the two general linear models, model 1 with the components (Table 4) and model 2 with the individual variables (Table 5) shows that only the variables with the highest score on a component in the PCA, i.e. herd leg hygiene score on component 1 and prevalence of cows with nodes on component 2, were related to an increase in CBSCC. These two variables explained nearly as much of the variation in CBSCC as the components.

Component 1 indicates that there are differences between breeds. Whether these differences are due to breed characteristics or management and attitudes associated with keeping a specific breed has to be further explored.

We could not find an association between mastitis treatments per cow year and the prevalence of cows with dry quarters. Both variables were only related to CBSCC in combination with other variables as a component, but not as individual variables.

The herd leg hygiene score is a direct measurement of the level of a risk factor for having high CBSCC and has potentials in udder health assessment.

Component 2 consists of variables that indicate a pathological change in the udder quarter. The prevalence of dry quarters depends on the farmer's threshold when to treat or blind a quarter of a cow with mastitis. This may be influenced by the level of mastitis in the herd and the severity of symptoms. Regardless of the incidence of clinical mastitis, farmers, which dry off single quarters efficiently at an early stage, may have a low CBSCC, because the blinded quarter does not contribute to the CBSCC. The prevalence of dry quarters is mainly a result of a management decision, similar to the decision-making process regarding mastitis treatments as described by Vaarst and others (2002). The prevalence of cows with nodes is a direct measurement of the cow's response to mastitis and is to a lesser degree dependent on management decisions.

Conclusions

PCA extracted two components that may represent two different herd characteristics: the 'Dirty, DH & treatments' type and the chronic udder type that were positively related with CBSCC. Herd leg hygiene score (within component 1) and prevalence of cows with nodes (within component 2) were associated with CBSCC, explained alone as much of the variation in CBSCC as the components and are therefore important udder health indicators. Mastitis treatments, prevalence of dry quarters and CBSCC were not related, which indicated that a low usage of antibiotics did not have a negative effect on udder health. Herds with Jersey had the lowest usage of antibiotics for mastitis without large problems with elevated CBSCC. The Jersey herds also had the cleanest cows, but it was not possible to decide whether these results were due to management or breed effects.

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